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14. ABSTRACT
Operation Brain Trauma Therapy (OBTT) is a multi-center pre-clinical drug screening consortium testing therapies in multiple animal models of traumatic brain injury (TBI) with the goal of identifying the best therapies for clinical trials. OBTT is also evaluating serum biomarkers of TBI across its models. Screening of therapies is carried out at three sites using three distinct rat models, i.e., parasagittal fluid percussion injury (FPI, University of Miami), controlled cortical impact (CCI, University of Pittsburgh), and penetrating ballistic-like brain injury (PBBI, WRAIR). The serum biomarkers GFAP and UCH-L1 are being serially assessed in all models (Banyan). Biomarker development is also ongoing in a large animal (micropig) FPI model (Virginia Commonwealth University) with parallel studies. The most promising drug will be tested in the micropig model to advance a therapy up the phylogenic scale. OBTT's work is ongoing as it completes year 3 of operation. OBTT is currently screening its 6th drug, has studied >900 rats, and assessed ~3000 biomarker samples.

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INTRODUCTION

As outlined in the grant proposal and in the prior progress reports, Operation Brain Trauma Therapy (OBTT) is a unique multi-center, pre-clinical, drug screening and brain injury biomarker development consortium for the ultimate translation of the best potential drugs to clinical trials in traumatic brain injury (TBI, **Figure 1**). OBTT includes investigators at the Safar Center for Resuscitation Research (Univ. of Pittsburgh School of Medicine, Patrick Kochanek, PI; C. Edward Dixon, Co-I), the Miami Project to Cure Paralysis, (Univ. of Miami School of Medicine, W. Dalton Dietrich, site PI; Helen Bramlett, Co-I), the Neuroprotection program at WRAIR (Frank Tortella, site PI; Deborah Shear and Kara Schmid, Co-Is), Virginia Commonwealth Univ. (John Povlishock, site PI) and Banyan
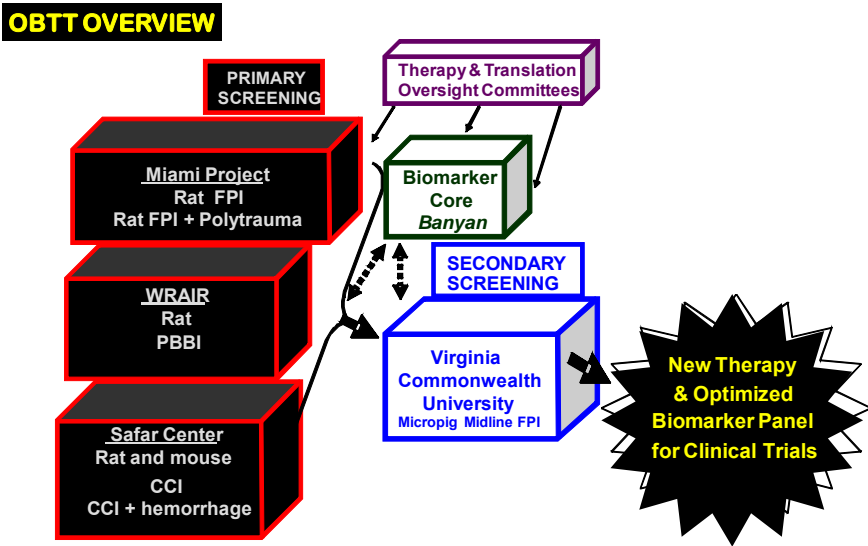

Figure 1. Schematic of the components and goals of OBTT.

Biomarkers (Ronald Hayes, site PI), Kevin Wang, (University of Florida), and Stefania Mondello (Messina University). Three rodent models (controlled cortical impact [CCI], parasagittal fluid percussion injury [FPI], and penetrating ballistic-like brain injury [PBBI]) are used in Pittsburgh, Miami, and WRAIR, respectively, for primary drug screening with the most promising candidates tested in a micropig TBI model at Virginia Commonwealth Univ. Additional screening of promising drugs is also carried out in more complex rodent models or with advanced monitoring, as appropriate. **The overall hypothesis is that clinical TBI is a heterogeneous disease involving multiple brain injury phenotypes and that success of an agent tested across multiple established TBI models using an approach with unprecedented rigor and blinding across centers will identify the best candidates for clinical trials.** Two types of drugs are screened, 1) low hanging fruit (drugs already FDA approved for other uses, or otherwise ready for clinical translation) and 2) higher risk but potentially high reward novel therapies. Drugs in the latter category should have at least some track record of success in experimental brain injury.
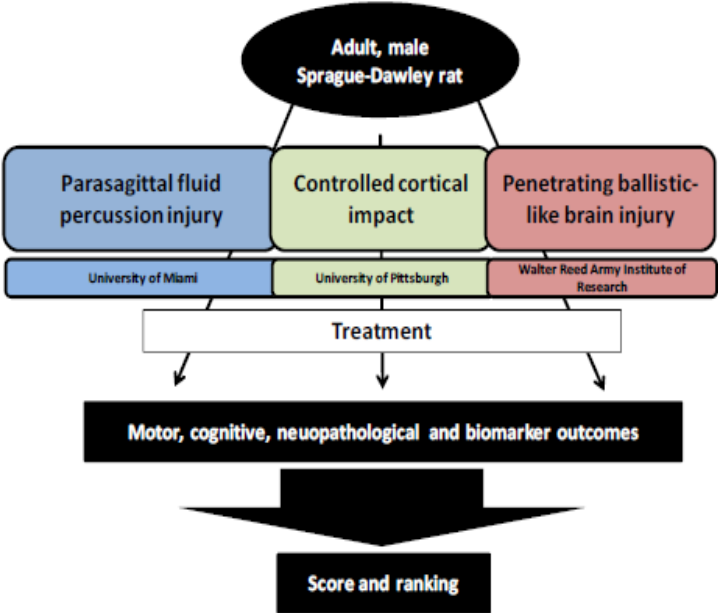

Figure 2. Primary Screening of therapies in established rat models of TBI.

BODY

Administrative overview of accomplishments in year 4 of funding:
Safar Center for Resuscitation Research (Patrick M. Kochanek, MD, overall PI)

Year 4 continued to be highly productive for OBTT. We identified a

drug with considerable benefit in 2 of our 3 screening models, namely, Levetiracetam. Our approach to primary screening of therapies is shown in **Figure 2**. The first 4 therapies (Nicotinamide, Erythropoietin [EPO], Cyclosporine-A [CsA], and Simvastatin), despite considerable literature support were unimpressive. It is noteworthy that EPO was just shown to be ineffective in severe TBI in a clinical trial—consistent with the findings of OBTT. **In contrast, drug #5, Levetiracetam (Keppra), showed significant benefit in 2 of the 3 screening models which is an exciting development in OBTT**. It is also the first drug tested by OBTT to show benefit on cognitive outcome in any of the screening models using our blinded and highly rigorous approach. Drug #6, Glibenclamide (glyburide) is also showing some benefit in FPI and CCI although the code has not been broken for all of the outcomes yet. We have thus far studied 956 rats and 33 micropigs and we have also nearly completed testing on the 7th therapy, the purported membrane re-sealing agent Kollidon-VA 64 and we are beginning testing on the 8th therapy, minocycline—after having carried out preliminary pharmacokinetic (PK) studies to define dosing. **We have also collected and assessed 3000+ serum and/or plasma biomarker samples across the models and treatments, and the results from the biomarker studies are quite impressive, particularly for GFAP and show strong correlations with both behavioral and histological outcomes across all of the TBI models.** We also selected several drugs (NIM 811, Edaravone, Amantadine, and Etanercept) for studies in the upcoming year, as time and resources permit. A comprehensive review of published studies is assembled for each drug (available upon request in the Manual of Standard Operating Procedure). Therapy selection has taken place each year at an annual site PIs meeting at the Congress of the National Neurotrauma Society (NNTS). Each dosing plan is developed based on the literature review. For each agent, in general, 4 experimental groups have been used in primary screening (sham, injury plus vehicle, and injury plus treatment at two different doses). The Morris

Table 1. Scoring matrix for assessment of therapeutic efficacy across models in OBTT					
( ) = point value for each outcome within each model					
Site	Neuro Exam	Motor	Cognitive	Neuropathology	Serum biomarker
Miami	None	Cylinder (2) Gridwalk (2)	Hidden platform latency (2) Hidden platform pathlength (2) MWM probe (2) Working memory latency (2) Working memory pathlength (2)	Lesion volume (2) Cortical volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)
Miami total	N/A	4	10	4	4
Miami Drug # Dose 1 Dose 2					
Pittsburgh	None	Beam balance (2) Beam walk (2)	Hidden platform latency (5) MWM probe (5)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)
Pittsburgh total	N/A	4	10	4	4
Pittsburgh Drug # Dose 1 Dose 2					
WRAIR	Neuroscore	Rotarod (3)	Hidden platform latency (5) MWM probe (3) Thigmotaxis (2)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)
WRAIR total	1	3	10	4	4
WRAIR Drug # Dose 1 Dose 2					
Grand total					
Drug # Dose 1 Dose 2					

MWM = Morris water maze; WRAIR=Walter Reed Army Institute of Research; GFAP = glial fibrillary acidic protein, UCH-L1 = Ubiquitin carboxy-terminal hydrolase L1; Δ = delta; N/A = not applicable

Table 1 COMMENT: Outcome scoring matrix used in primary screening in rat studies in OBTT. Each therapy tested can generate a maximum of 22 points at each center and thus a 66 point total overall. Cognitive outcome is given the greatest weight given its importance to clinical outcomes for drug development. This scoring matrix has been refined from its original format as a result of the ongoing work by our consortium.

water maze (MWM) is used to assess cognitive outcome and is the primary outcome parameter across sites. Motor testing is also carried out at each site, but varies depending on the model. Lesion volume and

hemispheric and/or cortical tissue loss are also assessed at each site. However, the drug, dose, treatment regimen, and biomarker sampling are identical between sites. **Table 1** shows the outcome scoring matrix used across sites for scoring of each drug tested in Primary Screening in OBTT. Our work has garnered attention and positive review in the TBI field. We gave a platform presentation at the 2014 MHSRS Conference and will be featured in a panel at the 2015 NNTS Congress. Thus far 3 manuscripts and a remarkable 38 abstracts and National or International presentations have been presented by OBTT investigators (including 14 this year alone; see Reportable Outcomes). We are also working on 8 manuscripts to comprise a special invited issue of the *Journal of Neurotrauma* (in preparation) devoted to OBTT—4 are complete. This will continue the high visibility for OBTT reflecting respect for the rigor and quality of the work. As we were completing this report, we were notified by the Soc. of Critical Care Med. that our submitted abstract on Levetiracetam in the CCI model was selected as one of the top 10 abstracts (out of 1000 submitted) and will receive the Scientific Award at the Congress. This again highlights how the work of OBTT is being viewed by the scientific community.

   We also hold a monthly conference call with a representative from each site (we have held 43 calls since the inception of OBTT) and we have held a face-to-face investigators meeting each year, to select therapies. Dr. Kochanek also contributed to the Pharmacology working group led by COL Salzer and Dr. Ramon Diaz-Arrastia which led to a major publication in the *Journal of Neurotrauma* (see Reportable outcomes #26). Related to the literature reviews carried out for OBTT, Dr. Kochanek and his team at the Safar Center just completed a comprehensive review article for *Seminars in Neurology* on Emerging Therapies in TBI in a special issue on TBI that will be edited by COL Geoff Ling (see Reportable Outcomes #27).

   An overview of the models and sites involved in Primary Screening in OBTT was provided previously. **We have completed primary screening of nicotinamide, EPO, CsA, simvastatin, levetiracetam, and glibenclamide, are nearly finished with Kollidon-VA 64 and are launching minocycline.** A presentation of all data from all sites for all drugs tested is beyond the scope of this report. An overview of findings, to date, in primary screening is provided below. We provide the overall scoring matrix results where the code has been broken across sites and discuss the findings. We also provide the pooled analysis outcomes for each agent— these represent only a small fraction of the total outcomes for each drug. We present a more detailed picture of the findings for the drugs studied in the past year (levetiracetam and glibenclamide).

   Based on the success of OBTT, we were invited to expand

Table 2. Scoring matrix for assessment of therapeutic efficacy across models in OBTT ( ) = point value for each outcome within each model DRUG: Nicotinamide; Dose 1 = 50 mg/kg; Dose 2 = 500 mg/kg						Model and Overall Total
Site	Neuro Exam	Motor	Cognitive	Neuropathology	Serum biomarker	
Miami-FPI	None	Cylinder (2) Gridwalk (2)	Hidden platform latency (2) Hidden platform pathlength (2) MWM probe (2) Working memory latency (2) Working memory pathlength (2)	Lesion volume (2) Cortical volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Miami total	N/A	4	10	4	4	
Miami Dose 1 Dose 2		0,0 0,0	0,0,0,0 0,0,0,+1,0	0,0 0,0	-0.5,0,0,0 0,-0.5,0,0	-0.5 +0.5
Pittsburgh-CCI	None	Beam balance (2) Beam walk (2)	Hidden platform latency (5) MWM probe (5)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Pittsburgh total	N/A	4	10	4	4	
Pittsburgh Dose 1 Dose 2		0,0 +1,0	-2.5,0 0,0	0,0 0,+2	0,0,0,0 +0.5,+0.5,0,0	-2.5 +4.0
WRAIR-PBBI	Neuroscore	Rotarod (3)	Hidden platform latency (5) MWM probe (3) Thigmotaxis (2)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
WRAIR total	1	3	10	4	4	
WRAIR Dose 1 Dose 2	0 0	0 0	0,0,0 0,0,0	0,0 0,0	0,0,-0.5,0 +0.5,+0.5,-0.5,0	-0.5 +0.5
Grand total Dose 1 Dose 2	0 0	0 +1	-2.5 +1.0	0 +2	-1 +1.0	-3.5 +5.0

Overall Nicotinamide
Low dose: -3.5
High dose: + 5.0
Largest positive model effect in CCI +4.0 for high dose

Table 2 COMMENT: Scoring matrix results for Drug 1, nicotinamide after primary screening in rats in OBTT. Nicotinamide, had only a modest benefit only at high dose. It was seen largely in the CCI model and the greatest contributor to that effect was tissue sparing. Benefits on cognitive outcome were sparse–with negative effects at low dose and a benefit at high dose on only 1 outcome (working memory) in only one model (FPI).

our work. We submitted a proposal titled "OBTT Extended Studies (OBTT-ES)" and it was just funded. The goal of OBTT-ES is to test more high risk agents than in OBTT. We are about to begin studies across the consortium with the aquaporin 4 blocker AER-271. The findings of OBTT-ES will be discussed in a separate report since it represents a separate grant.

Drug 1: Nicotinamide: Nicotinamide (Vitamin B3) has shown dramatic benefit on function, pathology, and blood-brain barrier damage, with several positive reports in TBI, including CCI and FPI. Most reports showing benefit of nicotinamide in TBI are from a single laboratory. It has shown benefit on several mechanisms including poly-ADP-ribose polymerase activation, inflammation, and replenishing NADPH levels. Doses of 50-500 mg/kg have shown efficacy and with a promising 4 h time window. Nicotinamide is commercially available. It is an example of an agent that could be readily moved forward if found to show benefit and could also be used as a nutritional supplement in a pre-treatment approach in light of the ability to provide dietary neuroprotective additives in theater. References on nicotinamide were presented in last year's report.

Treatment or vehicle was given at 15 min and 24 h IV after injury--and this approach was used at all of
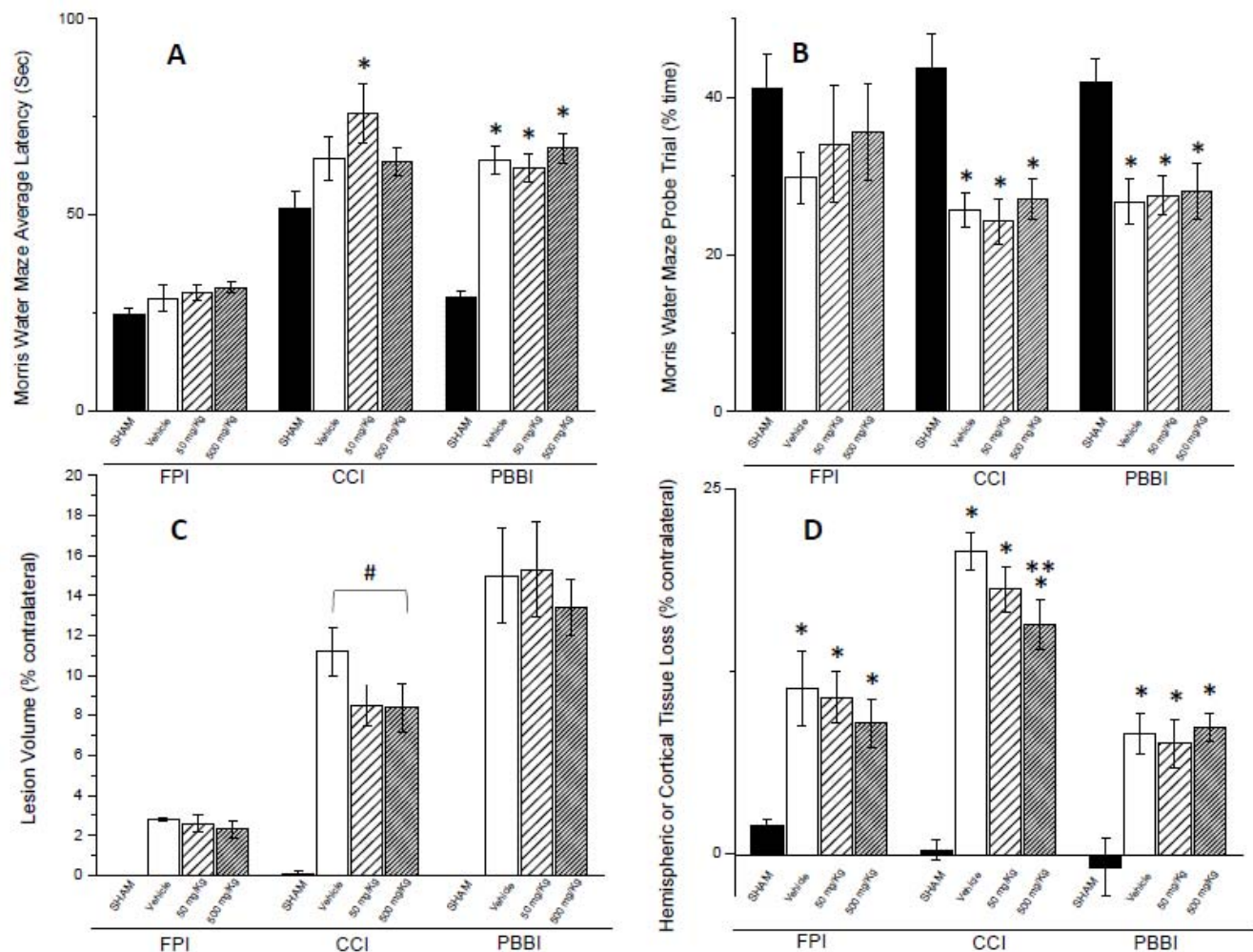

Figure 3 A-D. Montage of 4 outcomes in screening of nicotinamide in OBTT in the FPI model (Miami) the CCI model (Pittsburgh) and PBBI model (WRAIR). These graphs provide an overview of drug effects across models and show the level of severity produced by each model. (A) Deficit in MWM performance in the hidden platform paradigm was modest in FPI and more substantial in CCI and PBBI. Surprisingly, low dose nicotinamide worsened MWM latency in CCI. (B) Probe trial showed again a modest deficit on the FPI model with trends toward benefit from nicotinamide. In CCI and PBBI robust deficits were seen, but neither dose improved performance. (C) Damage was modest in FPI vs. CCI or PBBI as assessed by lesion volume. There was a trend toward reduced lesion volume for both doses in CCI ($^{\#}P$ = 0.07). **(D)** Tissue loss normalized for contralateral cortical volume in FPI and for hemispheric volume in CCI and PBBI showed **a significant reduction in CCI (**$^{**}P$ <0.05 for high dose vs. vehicle).** A trend toward reduced tissue loss was also seen in the FPI model. **This approach is being used for all drugs in OBTT.**

the primary screening centers. Low dose (dose 1) was 50 mg/kg while high dose (dose 2) was 500 mg/kg. In summary overall low dose nicotinamide at 50 mg/kg was not effective (**Table 2** and **Figure 3A-D**). In contrast, and in part consistent with the literature, nicotinamide at high dose (500 mg/kg) produced some modest benefit

including benefit on working memory in the FPI model, tissue sparing and modest motor benefit in the CCI model, and a benefit on the serum biomarker GFAP in PBBI. Figure 3 summarizes the key findings across models. The largest effect was its effect on tissue sparing which was significant in CCI and showed trends in FPI. Parallel to those findings, we saw a significant reduction of serum GFAP in PBBI and with trends in CCI suggesting that biomarkers may have potential to assess tissue loss. Disappointing, however, was the lack of benefit on cognitive function across models. Looking back at the literature on nicotinamide, tissue sparing is the most consistently reported finding along with benefit on motor function; cognitive outcome benefits are rarely reported. Our data suggest that the literature may be somewhat overstated, although our findings were in the same direction. Our data also suggest that biomarkers, notably GFAP performed extremely well and are worthy of continued use in our OBTT design. Our data also suggest that with tissue sparing in some models, it might be useful in combination therapy--which we are considering as an option in future years if no single therapy appears highly effective. For example, nicotinamide plus a cognitive enhancing agent might be logical.

Drug #2, EPO: The *PubMed* literature search carried out before launching our cross model screening studies in OBTT revealed a total of 28 publications showing benefit of EPO or its analogs in rodent models of TBI and identified an ongoing single center clinical trial.[1-28] A pleiotropic cytokine involved in erythropoiesis, EPO has many effects that could be important in TBI such as anti-excitotoxic, anti-apoptotic, antioxidant, and anti-inflammatory actions, stimulation of neurogenesis and angiogenesis, and protection of mitochondria, among others. The exact mechanism or mechanisms of benefit are unclear. Although classical EPO receptors are seen in many cell types in the CNS, they are up-regulated by hypoxia, and EPO receptor null mice have a worse outcome than wt after CCI, surprisingly, the literature suggests that EPO receptors are not required to mediate the benefit of exogenously administered EPO in preclinical models of TBI.

Table 3. Scoring matrix for assessment of therapeutic efficacy across models in OBTT ( ) = point value for each outcome within each model DRUG: EPO; Dose 1 = 5000 IU/Kg; Dose 2 = 10,000 IU/Kg						Model and Overall Total
Site	Neuro Exam	Motor	Cognitive	Neuropathology	Serum biomarker	
Miami	None	Cylinder (2) Gridwalk (2)	Hidden platform latency (2) Hidden platform pathlength (2) MWM probe (2) Working memory latency (2) Working memory pathlength (2)	Lesion volume (2) Cortical volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Miami total	N/A	4	10	4	4	
Miami Dose 1 Dose 2		0,0 0,0	0,0,0*,0 0,0,0,0	0,0 0,0	0,0,0,0 0,0,0,0	0 0
Pittsburgh	None	Beam balance (2) Beam walk (2)	Hidden platform latency (5) MWM probe (5)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Pittsburgh total	N/A	4	10	4	4	
Pittsburgh Dose 1 Dose 2		0,0 +1,0	-2.5,0 -2.5,0	0,0 0,0	0,0,0,0 0,0,0**,0	-2.5 -1.5
WRAIR	Neuroscore	Rotorod (3)	Hidden platform latency (5) MWM probe (3) Thigmotaxis (2)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
WRAIR total	1	3	10	4	4	
WRAIR Dose 1 Dose 2	0 0	0 0	0,+1.5,0 0,+1.5,0	-2,0 0,0	0,0,0,0 0,0,0,0	-0.5 +1.5
Grand total						
Dose 1	0	0	-1.0	-2	0	-3.0
Dose 2	0	+1	-1.0	0	0	0

*Working memory ANOVA P=0.037, post hoc NS—trend toward worse in low dose
**High dose P=0.05 for a reduction in UCH-L1 vs. vehicle at 24 h

Overall EPO
Low dose: -3.0
High dose: 0
Largest positive model effect in PBBI +1.5 for high dose

Table 3 COMMENT: Scoring matrix results for Drug 2, EPO. EPO was remarkably devoid of benefit across the primary screening models used in OBTT compared to the many supportive studies in the literature. However, our findings in OBTT are consistent with the recent randomized controlled clinical trial which showed no benefit in severe TBI.

administered EPO in preclinical models of TBI.   Species included rats and mice and models included CCI, FPI, impact acceleration, closed head injury, Feeney weight drop, and TBI plus hemorrhage. Studies in large animal models, however, were not identified. **Based on the literature, we chose doses of 5000 U/kg and 10,000 U/kg as a single IV bolus at 15 min after TBI.** 5000 U/kg had the most support. Therapeutic window is controversial; some studies suggest benefit with first dose as late as 24 h. The most complete study of time

window identified 6 h as the latest effective time point. **Again, at each site, we used 4 groups, sham, TBI + vehicle, and TBI + treatment at low and high doses** with a sample size of ~10 rats per group in each model.

The overall scoring sheet for EPO is provided in Table 3 and the pooled analysis outcomes from primary screening are shown in Figure 4A-D. Surprisingly, we did not detect meaningful benefit of EPO
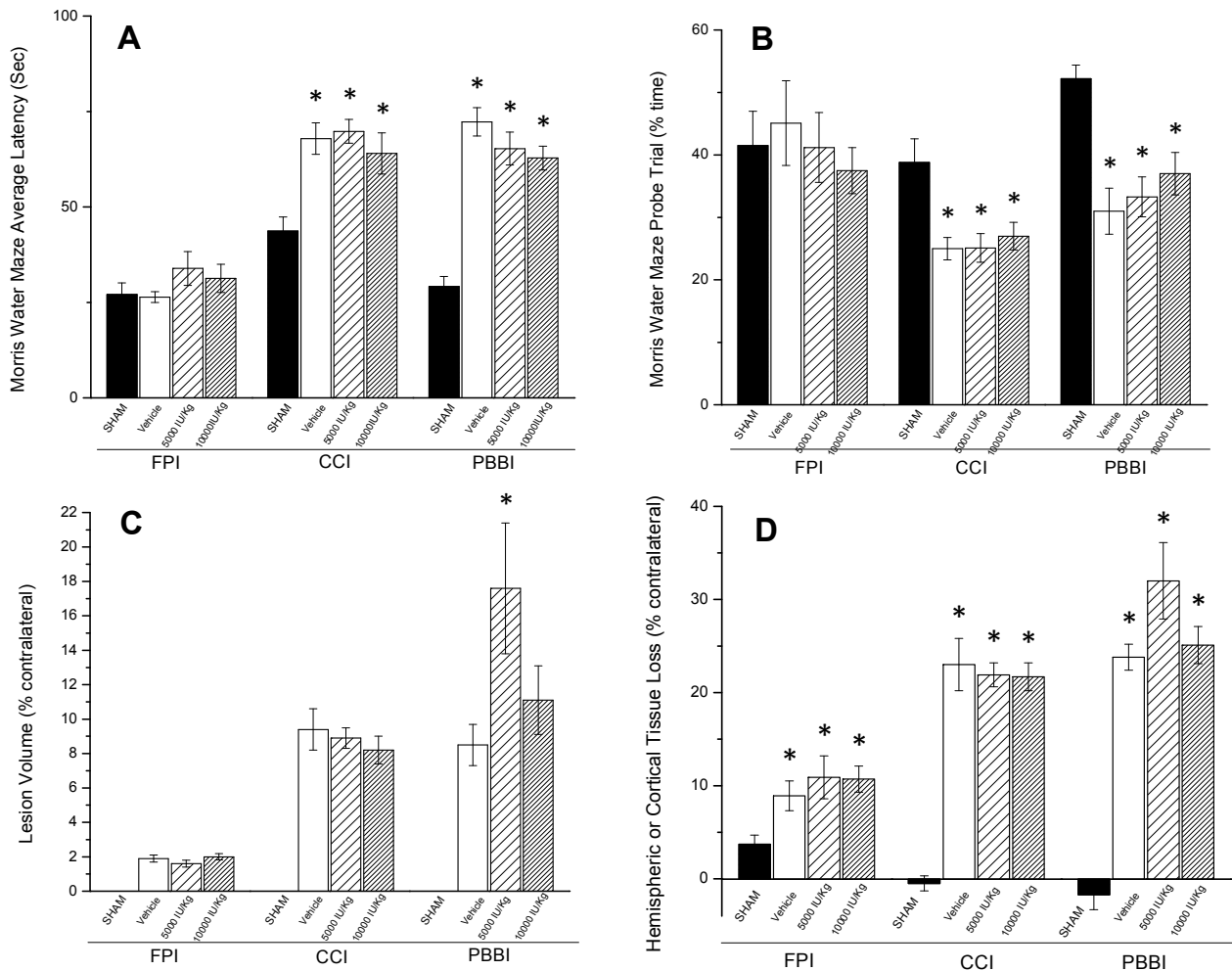

Figure 4 A-D. Montage of 4 outcomes in screening of EPO in OBTT. (A) Surprisingly, EPO had no effect on MWM performance across models. (B) Probe trial showed a modest deficit in FPI with no benefit in CCI or PBBI. (C) Low dose EPO expanded the lesion in the PBBI model (P<0.05) vs. vehicle. **(D)** Tissue loss showed no benefit from EPO across models.

across any of the outcomes in the OBTT consortium in primary screening studies. This was surprising given the many positive reports. However, recent reviews from the field of cancer suggest that often the literature on pre-clinical work is over-inflated –even when positive studies are seen in several independent laboratories (see Begley, *Nature* 2012). The rigor of OBTT may circumvent this problem. It is also possible that we underestimated the potential benefit of EPO by using only a single post injury dose in our approach across the OBTT consortium, however, some prior studies have shown benefit from the identical approach used in OBTT and it also has been reported that multiple doses of EPO can produce polycythemia and hyperviscosity and increase thrombosis which we wished to avoid given the detrimental effects of EPO seen in stroke trials—which are believed to be related to these side effects. **Our negative findings with EPO are consistent with the findings of the recent randomized controlled clinical trial showing no benefit of EPO therapy in patients with severe TBI.[81] Thus the findings of OBTT are consistent with the clinical trial.**

Drug #3. CsA: CsA is in widespread clinical use as an immunosuppressant. Inhibition of mitochondrial permeability transition pore opening is suggested to confer benefit in TBI by preserving mitochondrial function and reducing oxidative stress. Calcineurin inhibition may also benefit learning/memory by blocking its phosphatase activity. Immunosuppressive effects, also mediated by calcineurin inhibition, may also confer benefit (or side effects). 17 studies in pre-clinical TBI models were identified on *PubMed* (16 positive) prior to

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the selection of CsA by OBTT.[29-45] Multiple histological outcomes were benefited (axonal injury, lesion volume) in multiple labs. Surprisingly, there are few studies of CsA on behavior after TBI—two studies show benefit on motor outcomes, and one on MWM. Most studies were carried out in impact acceleration or CCI, with a few in FPI. All but 3 were carried out in rats, with one in mice, piglets, and ewe. Most work was done in males. There are studies of dose response, route of administration, therapeutic window, and brain tissue levels. IV dosing is preferred in OBTT rather than IP for reasons of clinical translation; the IV route is available for CsA. Early work showed limited BBB passage. While that is true in uninjured brain, data in impact acceleration in rats show that brain tissue levels after 20 mg/kg mirror those seen after a 10 mg/kg intrathecal dose. Most studies show efficacy with 10-20 mg/kg. The only study showing benefit on cognitive outcome used low doses of 0.675 mg/kg or 18.75 mg/kg. In other studies, 1 or 3 mg/kg were of little efficacy on histology. High doses of 150 mg/kg were also not effective. Therapeutic window studies suggest that 15 min is better than 1h with some efficacy to 8h. Some studies used a second dose at 24 h.

Given all of this information, we tested 10 or 20 mg/kg IV infused over 5 min at 15 min and 24 h after injury across models in OBTT. The overall scoring sheet for CsA is provided in Table 4 and other outcomes are shown in Figure 5A-D (below). Unlike other drugs tested thus far, CsA as used in OBTT showed great model dependence with some benefit in the mildest insult (FPI) but toxicity in models with the most severe injury (CCI and PBBI).

Specifically, CsA showed some benefit in the mildest insult FPI, but modest deleterious effects on motor and cognitive function in CCI and mild toxicity (2 rats died; 2 had seizures). In the most severe model, PBBI, both CsA and its vehicle (cremophor) showed considerable toxicity at high dose (29% mortality and similar mortality in the vehicle group) and no benefit on any outcome. Presentation of all of the data is beyond the scope of this report. Lack of a benefit on tissue sparing in CCI and deleterious effects on behavior were surprising. However, it is not well recognized, that few pre-clinical studies have assessed behavioral outcomes with CsA in TBI. Toxicity in PBBI was unanticipated. One possibility is that we used IV dosing, contrasting the IP dosing in most other work in rats. IP absorption of CsA is erratic and it is possible that high levels show toxicity in models where there is a high level of BBB permeability—i.e., the most severe models. **Our work also suggests that differences between patients in the amount of BBB injury might make the response to CsA treatment variable—**

Table 4. Scoring matrix for assessment of therapeutic efficacy across models in OBTT
( ) = point value for each outcome within each model
DRUG: CsA; Dose 1 = 10 mg/Kg; Dose 2 = 20 mg/Kg*

Site	Neuro Exam	Motor	Cognitive	Neuropathology	Serum biomarker	Model and Overall Total
Miami	None	Cylinder (2) Gridwalk (2)	Hidden platform latency (2) Hidden platform pathlength (2) MWM probe (2) Working memory latency (2) Working memory pathlength (2)	Lesion volume (2) Cortical volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Miami total	N/A	4	10	4	4	
Miami Dose 1 Dose 2		0,0 0,0	0,1,0,0,0 0,1,0,0,-1	0,2 0,0	0,0,0,0 0,0,0,0	3 0
Pittsburgh	None	Beam balance (2) Beam walk (2)	Hidden platform latency (5) MWM probe (5)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Pittsburgh total	N/A	4	10	4	4	
Pittsburgh Dose 1 Dose 2		-1,0 -1,0	0,0 -2.5,0	0,0 0,0	0,0,0,0 0,0,0,0	-1 -3.5
WRAIR	Neuroscore	Rotarod (3)	Hidden platform latency (5) MWM probe (3) Thigmotaxis (2)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
WRAIR total	1	3	10	4	4	
WRAIR Dose 1 Dose 2	0 0	0 0	0,0,0 0,0,0	0,0 0,0	0,0,0,0 0,0,0,0	0 0
Grand total						
Dose 1 Dose 2	0 0	-1 -1	1.0 -2.5	2 0	0 0	2 -3.5

Toxicity and mortality was seen with the high dose and vehicle in the PBBI model

Overall CsA
Low dose: +2.0
High dose: -3.5
Largest positive model effect +3.0 for low dose in FPI

Table 4 COMMENT: Outcome scoring matrix results for Drug 3, CsA in primary screening in rats in OBTT. **CsA was unique thus far among drugs evaluated in OBTT in that its effects were highly model dependent.** In FPI, our mildest insult, CsA showed modest benefit at low dose, and no toxicity. In CCI, CsA showed modest deleterious effects at both doses and mild toxicity. In the more severe PBBI model, both CsA at high dose, and its vehicle (cremophor) were toxic and associated with an increase in mortality rate and showed no benefit on outcome in survivors. Please see text for details.

from benefit to toxicity, and could thus make this agent challenging to dose clinically. In PBBI, the vehicle was also toxic. Assessment of brain tissue levels across models could be helpful but beyond the scope of drug screening in OBTT.

Our findings suggest that some of the more novel analogs of CsA with potentially less toxicity, such as NIM 811 or Neurovive might have a better chance to show benefit with IV administration and might be logical
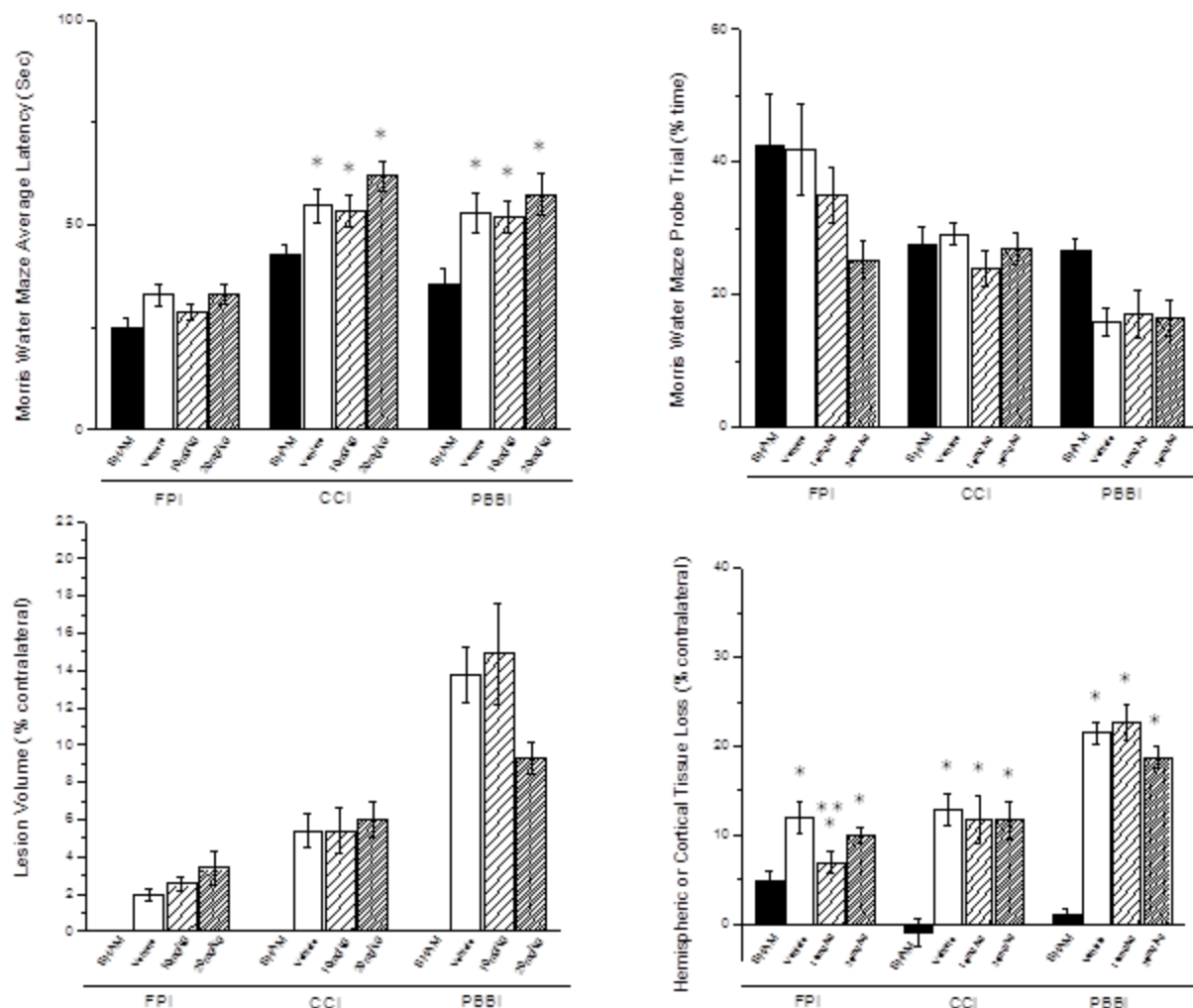

Figure 5A-D. Montage of 4 outcomes in screening of CsA in OBTT. (A) Deficit in MWM performance in the hidden platform paradigm was modest in FPI and more substantial in CCI and PBBI. Surprisingly, CsA had no effect on MWM performance across models. (B) Probe trial surprisingly showed no significant deficits across models, although considerable variability. (C) CsA had no effect on lesion volume although at low dose it reduced cortical tissue loss in the FPI model, consistent with its modest beneficial effects on cognitive function as shown in Table 4. No benefit on tissue sparing was seen in either CCI or PBBI despite excellent therapeutic targets for neuropathology.

to test. This could be particularly true in the setting of mild TBI—a finding which could be important to the Army. We mention this possibility given the fact that the only model with trend toward benefit in OBTT was our mildest model, namely, FPI. We will consider studying NIM 811, and have submitted a request to Novartis for this drug for OBTT; of note, they approved our request however, unlike with other drugs, obtaining a MTA with them to allow the study to move forward has been difficult. NIM 811 is a non-immunosuppressant CsA analog reported to potently reduce mitochondrial permeability transition pore opening. It might thus be promising in TBI as suggested in recent reports from the Sullivan group in Kentucky. The other agent, Neurovive is a CsA formulation that does not use the cremophor vehicle—and since the vehicle group showed toxicity in the PBBI model, we are also considering that agent in year 5 or possibly in OBTT-Extended Studies. **We believe that our studies with CsA exemplify the value of OBTT—namely, pointing out the critical importance of testing therapies in multiple models given the myriad TBI phenotypes and severities in humans. Abstracts #16, 18, 20, 22, 23, and 25 in Reportable Outcomes address our work on CsA.**

Drug #4. Simvastatin: The 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor Simvastatin reduces serum cholesterol but also inhibits neuro-inflammation and has possible effects on brain edema, Akt, CBF and trophic factor production.   A total of 15 studies were identified with Simvastatin in TBI prior to selecting it for OBTT.[54-68] Oral dosing reduced CA3 cell death and improved MWM performance after CCI in rats. MWM findings showed benefit on probe trial. Simvastatin showed greater benefit than Atorvastatin. Both are FDA approved and thus, low hanging fruit. Sierra et al[69] compared 9 statins with regard to their BBB penetration, HMG CoA reductase inhibition, and protection vs. neuro-degeneration from Tau and found Simvastatin to be best. Considering all of this information, Simvastatin was selected for testing by OBTT.

Mahmood et al[55, 56] reported benefit of Simvastatin on motorscore after CCI in female Wistar rats. A reduction in CA3 cell death was also seen. Oral simvastatin also reduced TUNEL after CCI in rats.[58,59]

Table 5. Scoring matrix for assessment of therapeutic efficacy across models in OBTT ( ) = point value for each outcome within each model DRUG: Simvastatin; Dose 1 = 1 mg/kg; Dose 2 = 5 mg/kg						Model and Overall Total
Site	Neuro Exam	Motor	Cognitive	Neuropathology	Serum biomarker	
Miami-FPI	None	Cylinder (2) Gridwalk (2)	Hidden platform latency (2) Hidden platform pathlength (2) MWM probe (2) Working memory latency (2) Working memory pathlength (2)	Lesion volume (2) Cortical volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Miami total	N/A	4	10	4	4	
Miami Dose 1 Dose 2		0,+1 0,+1	-2,-2,0,0,0 0,0,0,0,0	0,0 0,-1		-3.0 0
Pittsburgh-CCI	None	Beam balance (2) Beam walk (2)	Hidden platform latency (5) MWM probe (5)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Pittsburgh total	N/A	4	10	4	4	
Pittsburgh Dose 1 Dose 2		0,0 +1,0	0,0 0,0	0,0 0,0		0 +1.0
WRAIR-PBBI	Neuroscore	Rotarod (3)	Hidden platform latency (5) MWM probe (3) Thigmotaxis (2)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
WRAIR total	1	3	10	4	4	
WRAIR Dose 1 Dose 2	0 0	+1.5 +1.5	0,0,0 0,0,0	0,0 0,0		+1.5 +1.5
Grand total						
Dose 1	0	+2.5	-4.0	0		-1.5
Dose 2	0	+3.5	0	-1		+2.5

Overall Simvastatin (preliminary, biomarker data under analysis)

Low dose -1.5
High Dose +2.5
Largest positive model effect +1.5 for either does, in PBBI

Table 5 COMMENT: Preliminary outcome scoring matrix results for Drug 4, Simvastatin after primary screening in rats in OBTT. This represents only the results of the behavior and neuropathology. The biomarker studies have been completed but analysis is ongoing. **Cross model benefit was seen on motor outcomes, but deleterious effects were seen on cognitive outcome and neuropathology in FPI and no benefit for either cognitive outcome or neuropathology in CCI or PBBI.**

Usually, a dose of 0.5 or 1 mg/kg daily beginning on d1 and continued for 14d was used; 1 mg/kg was usually best. Chen et al[60] used a weight drop model in rats and higher doses 37.5 mg/kg PO at 1h and 6h and reported benefit on Rotarod, cytokines and edema. Beziaud et al[66] also used 37.5 mg/kg at 1h and 6h after FPI in rats and noted benefit on edema, BBB, and inflammatory markers. Abrahamson et al[62] reported benefit of Simvastatin (3 mg/kg PO daily, first dose at 3 h post CCI) on probe trial, but no effect on MWM latency in mice modified to express human Aβ. Chauhan et al[63] studied CCI in mice using 2 mg/kg in feeds noting benefit on probe trial. Shear et al[68] at WRAIR studied IV Simvastatin in PBBI in rats at 0.001, 0.01, 0.1, and 1.0 mg/kg. They gave a 10 min IV infusion at 30 min and 6h post-PBBI, and every 24h to 10d (all IV). There was no benefit on Rotarod; however, it dose-dependently protected vs. cognitive deficits on MWM. Chronic IV treatment was needed. Not all studies with Simvastatin are positive. Chen et al[64] used the parasagittal FPI model in rats and doses of 25, 37.5, 50, 75 or 100 mg/kg PO at 1h and 6h after TBI and noted a reduction in edema, but no benefit on neuroscore, beam walking or lesion volume. Indraswari et al[65] reported that Simvastatin at 1 or 5 mg/kg PO did not improve Rotarod performance after closed head injury in mice. Simvastatin directly enhances LTP.[70] No pre-clinical studies included naïve controls treated with Simvastatin; thus it is unclear if the cognitive enhancement after TBI represents an effect specific for TBI or simply non-specific enhancement of cognitive function.

Regarding dose, timing, and route of administration, oral (gavage) dosing of between 1 and 3 mg/kg daily for up to 14 d has by far shown the most benefit in published studies. A dose of 0.5 mg/kg was less effective. Several studies used higher doses with variable results. Most oral dosing studies used 14d of

treatment. Given the mission of OBTT to test promising drugs using established regimens in multiple laboratories, we tested **oral gavage treatment with Simvastatin; 1 or 5 mg/kg PO with first dose at 3 h and daily dosing for 14 d.** This also allowed OBTT to evaluate a therapy given chronically, to determine if such an approach produced–as suggested in the literature—more robust effects than simply acute administration.

Table 5 (above) and Figure 6A-D (below) show the preliminary behavioral and neuropathological
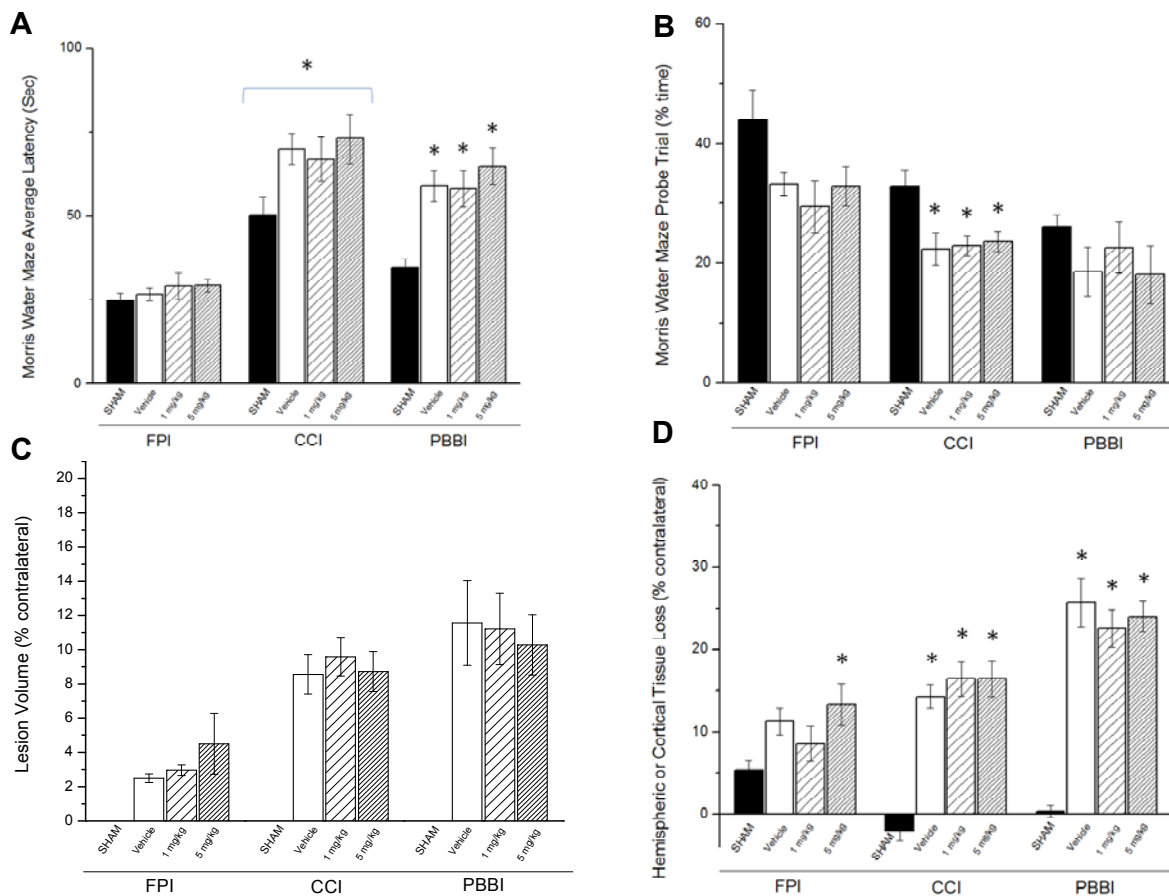
Figure 6A-D. Montage of 4 pooled analysis outcomes in screening of Simvastatin in OBTT. (A) Deficit in MWM performance in the hidden platform paradigm assessed as mean latency to find the hidden platform was not seen in FPI, but significant in CCI (*P<0.05 overall ANOVA) and PBBI (*P<0.05 vs. sham). However, no there was no effect of Simvastatin treatment on this outcome across models. (B) Probe trial showed surprisingly no significant deficit in either FPI or PBBI, but a significant deficit in CCI (P<0.05 vs. sham) that was again not influenced by treatment. (C) Simvastatin had no effect on lesion volume across modles, but (D) actually worsened cortical tissue loss at high dose in the FPI model (P<0.05 vs. sham). There was marked hemispheric tissue loss in CCI and PBBI (P<0.05 vs. sham) but no effect of Simvastatin.

outcomes across models for Simvastatin. **This was a demanding study for OBTT with 14 days of oral gavage administration carried out in each rat at each site.** As predicted by the literature, we noted some benefit on motor function across models. Indeed, Simvastatin represents the first therapy tested by OBTT to show some cross model benefit (in all 3 models) on any individual category of behavioral outcomes. However, the cross model benefit on various aspects of motor function was mild in magnitude with no outcome scoring full points. Disappointingly, we did not detect any benefit on cognitive outcome in any models. And in fact, MWM performance was actually worsened by treatment in the FPI model with full negative points for low dose in the MWM latency and pathlength. We also observed no benefit across models on histology. Although the final biomarker analysis is in progress, the findings certainly are not going to alter the overall conclusion with Simvastatin that it–at least using the literature supported oral treatment regimen will not be further pursued by OBTT. The WRAIR group has some positive effects with IV dosing in novel studies, and that may be worthy of pursuit. However, currently screening of other drugs by OBTT is taking precedence. Our findings with Simvastatin were recently presented at the NNT and MHSRS meetings (please see Reportable Outcomes).

Drug #5, Levetiracetam (Keppra): We identified 10 key references on Levetiracetam in TBI at the time of selection by OBTT.[71-80] It is a low hanging fruit for different reasons than most of the drugs considered by OBTT. It has a limited track record in experimental TBI, but has three compelling features 1) it targets posttraumatic seizures by unique mechanisms, 2) it has exceptionally low toxicity and 3) it is already in use in some clinical centers- although it is empiric and sporadic. It is believed to act at least in part via potentiation of GABAergic inhibition, although some non-GABA effects are operating. It inhibits burst firing without interference with normal neuronal excitability. It has a brain-specific binding site and selectively inhibits N-type Ca^{++} channels, exhibiting brain specific effects that differ from other anticonvulsants and thus confers anti-convulsant and excitotoxic effect with little apparent extracerebral toxicity except at extremely high drug doses. There are limited pre-clinical data supporting beneficial effects of Keppra in TBI. Wang et al[71] reported benefit in a murine CHI model with single IV doses of either 18 or 54 mg/kg at 30 min after injury and also in a second paradigm of SAH where treatment was given every 12 h for 3 d. In the CHI study, Levetiracetam was beneficial. Outcomes included Rotarod which was maximally benefited at 54 mg/kg and hippocampal cell death which was maximally benefited at 18 mg/kg (although the 54 mg/kg dose was similar). Fosphenytoin showed no benefit or detrimental effects. The rationale for testing Keppra in OBTT is thus related to several considerations 1) posttraumatic seizures, particularly with severe TBI are common (in patients) and sub-clinical status epilepticus worsens outcome in humans after severe TBI and is seen in our models, 2) the aforementioned pre-clinical study, 3) the fact that it is used by some centers as standard of care—while most use phenytoin, 4) the fact that there is controversy with regard to efficacy of fosphenytoin in TBI, particularly vs. Levetiracetam, and 5) there is recent pre-clinical data in TBI that suggests deleterious long-term effects of phenytoin therapy.[79] **Thus, our rationale for selection was that if we showed a clear benefit of Levetiracetam, it would suggest the need for a clinical trial that would likely be attractive and carried out.**

Regarding dosing, route of administration and PK, doses of 18 or 54 mg/kg IV 30 min after CHI in mice as a single dose was used in the only TBI study. The 54 mg/kg dose was at least if not more effective vs. the 18 mg/kg dose. Thus, we tested the 54 mg/kg dose and a higher dose.   A 15

Chemical Structure of Levetiracetam

Table 6.

Scoring matrix for assessment of therapeutic efficacy across models in OBTT ( ) = point value for each outcome within each model DRUG: Keppra Dose 1 = 54 mg/Kg; Dose 2 = 170 mg/Kg						Model and Overall Total
Site	Neuro Exam	Motor	Cognitive	Neuropathology	Serum biomarker	
Miami	None	Cylinder (2) Gridwalk (2)	Hidden platform latency (2) Hidden platform pathlength (2) MWM probe (2) Working memory latency (2) Working memory pathlength (2)	Lesion volume (2) Cortical volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Miami total	N/A	4	10	4	4	
Miami Dose 1 Dose 2		0,0 0,0	2,2,2,0,0 2,2,1,0,0	0,0 0,0	PENDING	6 5
Pittsburgh	None	Beam balance (2) Beam walk (2)	Hidden platform latency (5) MWM probe (5)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
Pittsburgh total	N/A	4	10	4	4	
Pittsburgh Dose 1 Dose 2		1,0 2,0	2.5,0 0,0	0,0 0,2	PENDING	3.5 4
WRAIR	Neuroscore	Rotarod (3)	Hidden platform latency (5) MWM probe (3) Thigmotaxis (2)	Lesion volume (2) Hemispheric volume (2)	GFAP 24 h (1) 4-24 h Δ (1) UCH-L1 24 h (1) 4-24 h Δ (1)	
WRAIR total	1	3	10	4	4	
WRAIR Dose 1 Dose 2	0 0	0 0	0,1.5,0 0,1.5,0	0,0 0,0	PENDING	1.5 1.5
Grand total Dose 1 Dose 2	0 0	1 2	10 6.5	0 2	PENDING	11 10.5

Overall Levetiracetam (preliminary, biomarker data under analysis)

Low dose +11
High Dose +10.5
Largest positive model effect +6 for low dose in FPI

Table 6 COMMENT: Preliminary outcome scoring matrix results for Drug 5 Levetiracetam after primary screening in rats in OBTT. Note that this represents only the results of the behavioral and neuropathology results across groups. The serum biomarker studies have been completed but the analysis is ongoing. **Overall significant benefit was seen in both the FPI and CCI models on multiple outcomes. Notable was that this agent is the first to show substantial benefit on cognitive outcome in any model (FPI) in this case, and it produced significant reduction of hemispheric tissue loss in the CCI model. Also, benefits were seen at both doses without a single negative point in any model for any outcome. This strongly suggests the need for further exploration of this agent either alone or in combination therapy in OBTT. Points from the biomarker findings could also further enhance its score.** Please see text for details.

min dosing regimen could be readily used. Data on PK in rats came from several studies. Loscher et al[72] studied daily IP injections of 13, 27, or 54 mg/kg for 21d. Seizure kindling was suppressed (1 h after dosing) but surprisingly seizure kindling was attenuated for >10d after the treatment was discontinued despite a 2-3h half-life in rats. Since a goal of OBTT is to replicate the best available studies, early, single dose administration with 54 mg/kg was used. The route of administration was IV, given the availability of an IV clinical formulation.

Other studies in rats suggest that doses higher than 54 mg/kg were worthy of exploring. Kiltgaard et al[74] studied a wide range of doses from 54 to 1700 mg/kg across many seizure paradigms such as NMDA, kainic acid, AMPA, bicuculline, picrotoxin, flumazenil, pilocarpine, and kindling. Efficacyvaried greatly depending on the inducing agent. For example, only 7 mg/kg IP abolished pilocarpine induced seizures, 54 mg/kg vs. kainite induced seizures, 97 mg/kg vs. DMCM-induced seizures, and 170 mg/kg vs. benzodiazepine antagonist-induced seizures. Levetiracetam was not effective against bicuculline- or picrotoxin-induced seizures. Thus, there is rationale for doses >54 mg/kg, and 170 mg/kg seemed logical, based on the literature. Toxicity was not seen in rats until doses of 1700 mg/kg twice daily were used; performance on Rotarod was impaired.
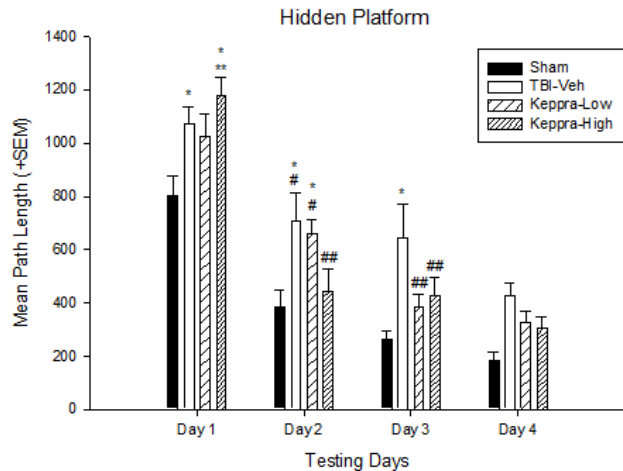

Figure 7. Benefit of Levetiracetam (Keppra) on cognitive assessment on hidden platform task (path length) in the FPI model. TBI-Vehicle (TBI-Veh) latency scores were higher than sham for all 4 d of testing. Two-way repeated measures ANOVA was significant for time (p<0.001) and group (p<0.001). Posthoc analysis for group was significant (p<0.05) for the following comparisons, TBI-Veh vs Sham, Keppra-Low and Keppra-High; Sham vs Keppra-Low, Keppra-High. (*vs Sham; ## vs TBI-Veh ;** Keppra-Low, # vs Keppra-High. Thus both dosages of drug are resulting in an iii
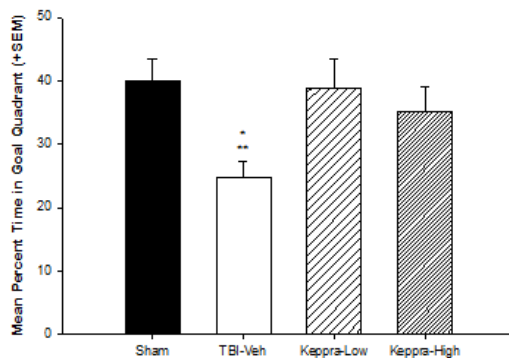

Figure 8. Example showing benefit of Levetiracetam (Keppra) on cognitive assessment on Probe Trial in the FPI model in OBTT. One-way ANOVA was significant for group (p=0.033). Posthoc analysis was significant (p<0.05) for TBI-Veh vs Sham(*) and Keppra-Low (**). Thus both doses resulted in an improvement in cognitive function with full points for low dose and partial points for high dose.

We thus used single 15 min post TBI dosing of either 54 or 170 mg/kg. This also limited the volume of fluid administered to 2 mL of saline (<10 mL/kg and thus clinically relevant). It was given as a single dose at 15 min after TBI.

The behavioral and histological data from OBTT on Levetiracetam show significant benefit in 2 of the 3 TBI models (FPI and CCI), with a slight benefit in the PBBI. The findings are shown in Table 6 and Figures 7-10 and are discussed below along with additional discussion in each of the respective figure legends. Detailed findings are presented for this agent because the studies of it were completed this year.

Overall significant benefit was seen in both the FPI and CCI models. Notable was that this agent is the first to show substantial benefit on cognitive outcome in any of the models in OBTT-- FPI in this case. It had multiple cognitive benefits in FPI including on hidden platform latency, pathlength and probe trial. It also produced motor benefit in CCI and a highly significant reduction of hemispheric tissue loss in CCI. The only benefit seen in PBBI was on probe trial. **Benefits were seen at both doses without a single negative point in any model for any outcome. This suggests the need for further exploration of this agent alone or in combination therapy in OBTT.** Points from the biomarker findings could further enhance its score; those samples have been collected and the analysis is pending.
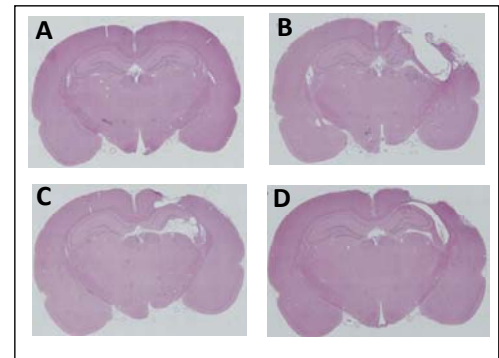

Figure 9. Representative (median animal in each case) coronal brain sections taken through the lesion from rats in the (A) sham, (B) TBI-Veh, (C) Keppra-Low and (D) Keppra-High dose groups after CCI. A significant reduction in hemispheric volume loss (P<0.05) was seen for both treatment groups vs. vehicle giving full points for this outcome to both Keppra treated groups. This represented a robust beneficial effect on histology for Keppra in CCI.

Given the success of Levetiracetam in OBTT, we felt that it was logical to provide a brief single graphical comparison of the effects of the first 5 drugs evaluated by OBTT. **Figure 11 (below) thus provides a comparison of behavioral and histological outcomes for the first 5 drugs in OBTT.** It is clear that Levetiracetam shows much more robust benefit than any of the other therapies tested thus far by our consortium; however, it is important to note that it still did not produce cognitive improvement across all models, and thus there is still a great deal of additional work to be done. **In any case, Levetiracetam has potential for clinical trial development and thus it will be tested this year in the more advanced murine model of CCI + hemorrhagic hypotension and it will also be tested in the micropig model at Virginia Commonwealth University by Dr. Povlishock and his group. It also has logical potential for testing of more sustained therapy (some work at WRAIR outside of OBTT has already done some investigations in that regard and has shown some promise). There may also be potential for this agent in combination**
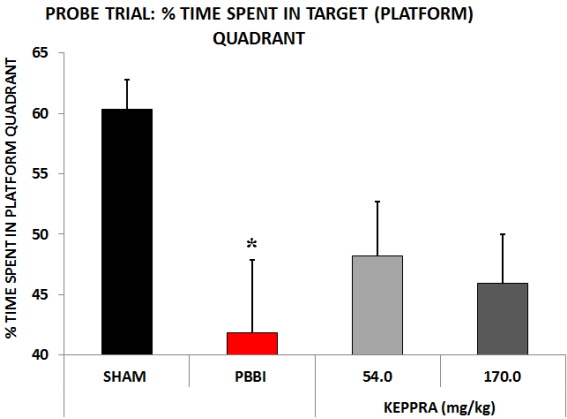
Figure 10. Benefit of Levetiracetam (Keppra) on cognitive assessment on Probe Trial in the PBBI model in OBTT. The PBBI group treated with vehicle (in red) was significantly different than sham but neither Keppra dose differed significantly from sham which indicated intermediate cognitive benefit for treatment on this task and resulted in partial points for both doses as shown in Table 6. * $P<0.05$ vs sham.
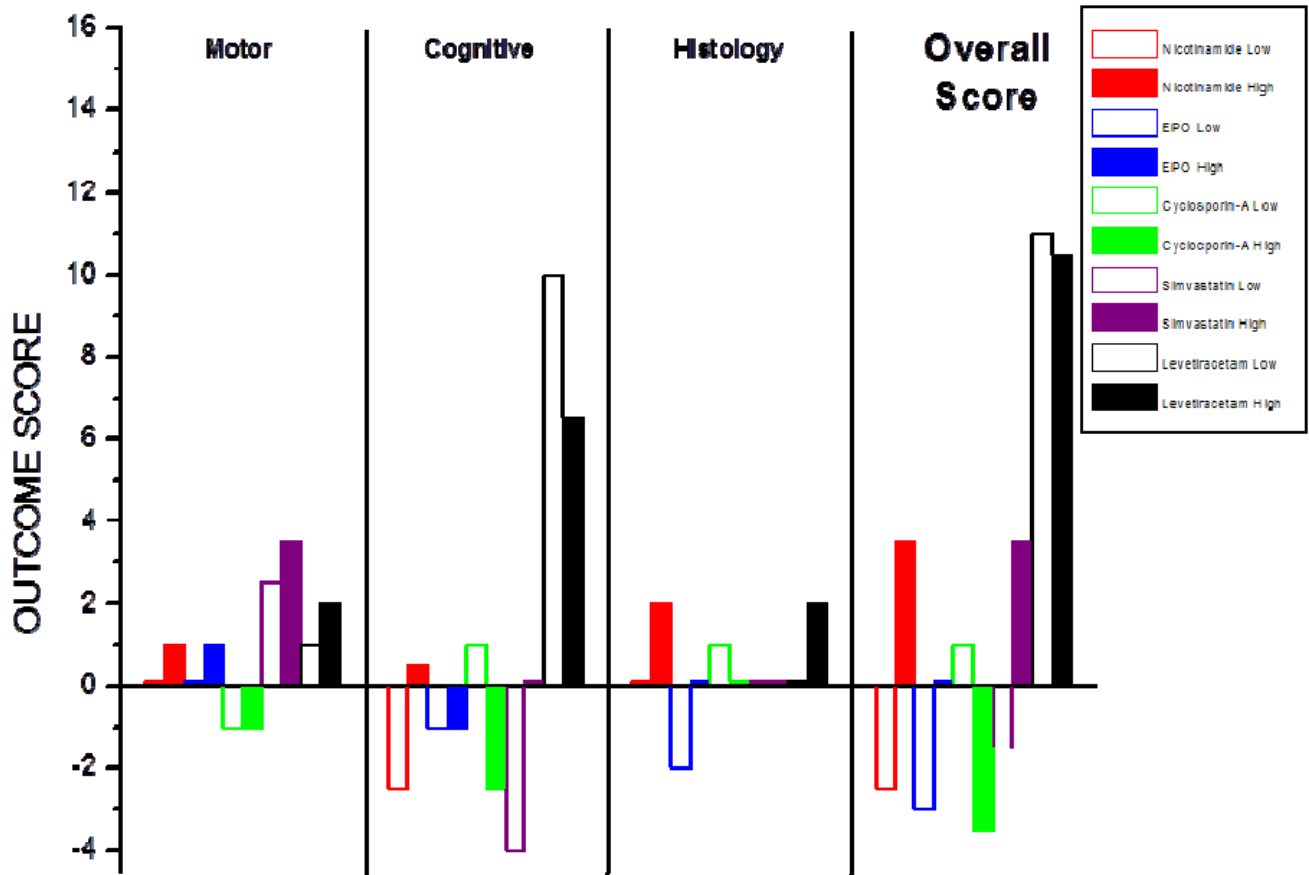

Figure 11. Comparison of the Motor, Cognitive, Histological, and Overall Score for the first 5 drugs in OBTT. These data are without the biomarker level, since those data from Simvastatin and Levetiracetam are still being analyzed. Levetiracetam has shown the most promise thus far and is being advanced to studies in the micropig model.

therapy. Based on its sporadic clinical use and safety record in TBI, it would also be reasonable to be tested in a clinical RCT, particularly given the potential liabilities of phenytoin, the current standard of care. All of these points will be discussed in the manuscript on our work with Levetiracetam in OBTT that is in preparation. In addition, all of the biomarker samples have been sent to Banyan and analyzed for both GFAP and UCH-L1 for this therapy and the data are being currently analyzed including assessment of treatment effects and correlations with behavior and neuropathology.

Drug# 6, Glibenclamide (Glyburide): Glibenclamide is a sulfonylurea receptor (SUR1) regulated NC_{Ca-ATP} channel antagonist that has shown promise in a number of studies in pre-clinical stroke models. A review of its use in CNS insults has been published.[82] The SUR1 channel is a nonselective cation channel (ABC binding cassette transporter) that
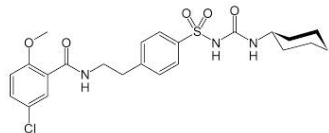
Chemical Structure of Glibenclamide (Glyburide)

is regulated by intracellular calcium and ATP. The ABC proteins couple ATP hydrolysis to translocation of solutes, xenobiotics or drugs across membranes. SUR1 activation leads to Na+ accumulation, cellular depolarization and ATP depletion. $SUR1NC_{Ca-ATP}$ channels are present in brain microvascular endothelium, neurons, and astrocytes, are induced by injury and by TNFα. Activation of this channel is associated with cell necrosis and cytotoxic edema. In addition to glibenclamide, this channel can also be blocked by the drug Riluzole which has also shown neuroprotective effects in pre-clinical studies.[83] However,
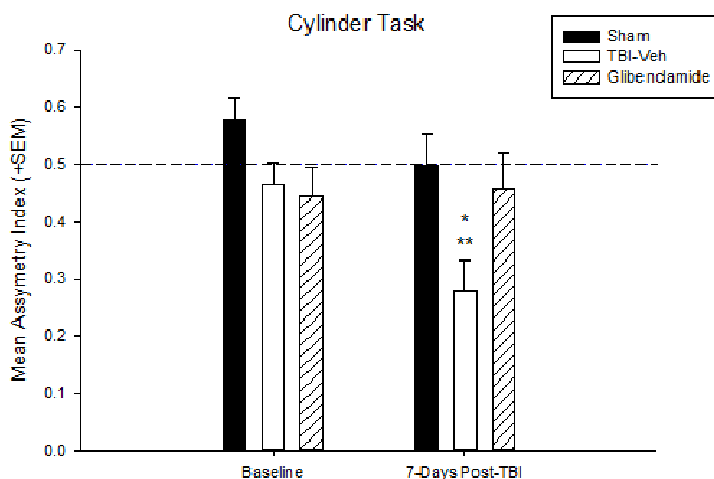

Figure 12. Effects of Glibenclamide on Cylinder Task in the FPI model. Rats performed equally on this task at baseline. After TBI, the TBI-Vehicle group was significantly impaired on this task vs. sham (*p<0.05) and Glibenclamide (**p<0.05) treated rats 7 d post-injury. Rats treated with Glibenclamide showed marked improvement on forelimb use. One-way ANOVA was significant for group (p=0.015). This produced full points for this task for treatment.

Glibenclamide is much more potent than Riluzole (EC_{50} of 48 nM vs. 31 µM, respectively). Also, SUR1 can be up-regulated by CNS injury. SUR1 upregulation was seen by 6h after CCI in rat hippocampus[84] and peaked at 12h and only partially resolved by 24h. Glibenclamide has shown promise in MCAO, thromboembolic models and malignant cerebral edema.[82, 85, 86] Reductions in infarct volume and mortality were seen with a 10h treatment window in MCAO.[96] It has also shown benefit in experimental SAH.[87] There have been reports in spinal cord injury models.[92] Most but not all have been positive depending on injury severity[88]—greater benefit in milder insults.

Two preclinical TBI studies in rat models have been published, both positive. Patel et al[84] studied Glyburide (10 µg/kg IP at 10 min after injury) followed by a SQ infusion of 200 ng/h for 7d by Alzet pump. This produced plasma levels of ~5 ng/mL with minimal effect on plasma glucose. Treatment reduced cleaved Caspase-3 in CA3 hippocampus and the number of Fluoro-Jade positive hilar neurons and improved probe trial. However, no benefit was seen on latency to find the platform in MWM at 2 wks after TBI. No motor data were presented. Hackenberg et al[89]; http://www.egms.de/static/en/meetings/dgnc2013/13dgnc397.shtml) studied glibenclamide in rat CCI using a SQ bolus 15 min after CCI and a 7d infusion. However, the exact dose was not described. Brain edema at 24h and contusion volume at 8 h, 24h and 7d, by MRI, were reduced.

There is an ongoing phase 2 clinical trial of IV Glyburide vs. placebo in TBI using MRI outcomes funded by the US Army via the INTRuST consortium. It includes patients across the injury spectrum; mild to severe (http://clinicaltrial.gov/ct2/show/NCT01454154?term=A+randomized+Clinical+Trial+of+glyburide+for+TBI&rank=1).

Finally, Glyburide is a sulfonylurea drug which can reduce blood glucose depending on the dose utilized. Fortunately, the doses that reduce blood glucose (producing hypoglycemia) in rats have been shown to be 30-400 times greater than those used in the neuroprotection studies and our studies in OBTT.[85] However, blunting of hyperglycemia that is seen in CNS insults could play some role in the observed benefit.

Regarding drug preparation, dosing and administration, the best characterized regimen and the one recommended in discussions with Dr. Simard from the University of Maryland come from his recent report.[86] In a model of MCAO stroke in rats, a 10 h therapeutic window was shown.

Thus far, in OBTT all of the injuries and treatments have been carried out for Glibenclamide. The results have shown some promise. We observed a significant benefit on motor function –full points for TBI
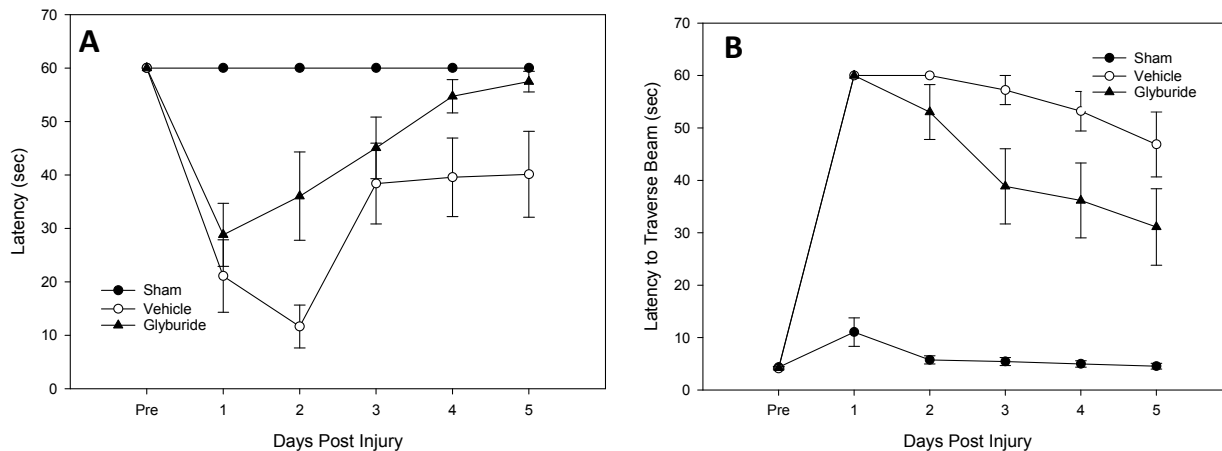

Figure 13A-B. Effect of Glibenclamide (Glyburide) on (A) beam balance and (B) beam walking motor tasks vs. vehicle treatment of rats after CCI. Glibenclamide treatment yielded beneficial (p<0.05) effects vs. vehicle resulting in full points for treatment for both motor tasks.

vehicle vs TBI glyburide treated in both the FPI and the CCI models. This is shown in **Figures 12 and 13**. Specifically, in the FPI model, significant benefit for glibenclamide treated rats was seen vs. vehicle after TBI on the cylinder task (**Figure 12**). In the CCI model highly significant benefit was seen for glibenclamide treated rats on both the beam balance and beam walking tasks (**Figure 13A-B**).   In contrast to FPI and CCI, no motor benefit was seen in the PBBI model—an example (Rotarod testing) is shown in **Figure 14**. However, benefit
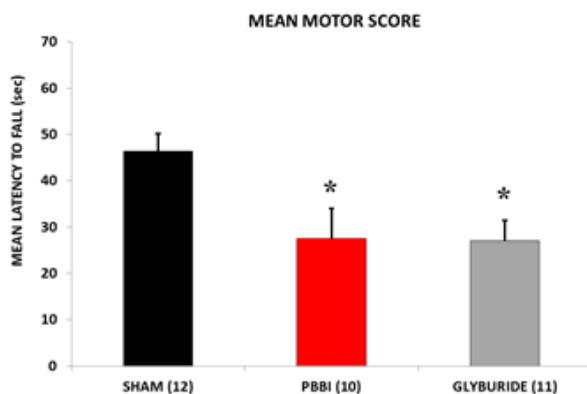

Figure 14. Example showing the effect of Glibenclamide on motor function (in this case Rotarod performance after TBI induced by PBBI. Unlike the FPI and CCI models, benefit of Glibenclamide on motor function was not seen in the PBBI model.

Figure 15. Example showing the lack of effect of Glibenclamide on cognitive outcome (MWM latency) after TBI induced by FPI. Contrasting benefit on motor function, no benefit was seen on MWM performance with Glibenclamide treatment. This same pattern was seen in the CCI model.

was not seen across models on cognitive outcome testing for MWM hidden platform paradigm, working memory, or probe trial (an example is shown in **Figure 15**). We have not yet broken the code on neuropathology for Glibenclamide although it is nearly completed at all of the centers. In addition, all of the biomarker samples have been sent to Banyan and analyzed for both GFAP and UCH-L1 for this therapy and the data are being currently analyzed including assessment of treatment effects and correlations with behavior and neuropathology. **Given that this drug targets brain edema, one**
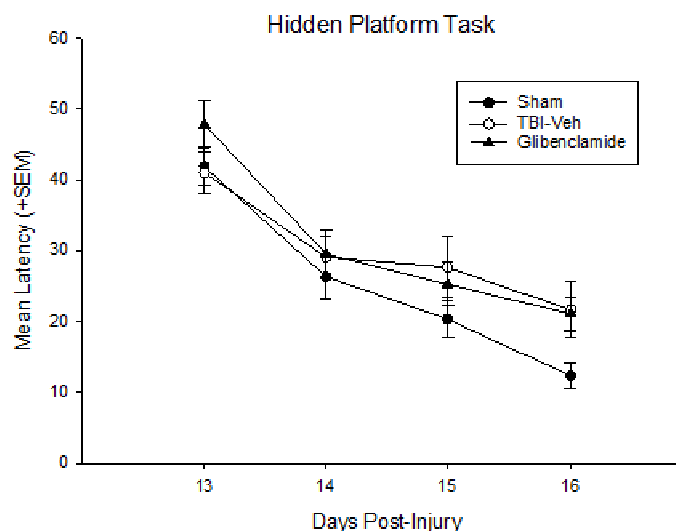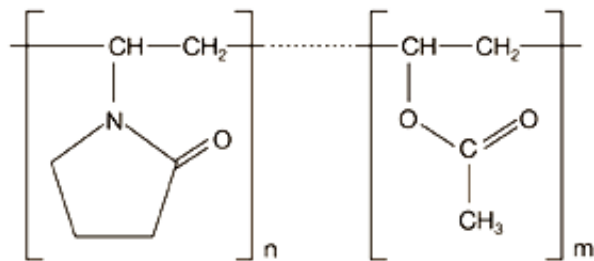
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possibility is that motor performance is improved because it is tested in the acute phase (during the initial week after injury) when brain edema peaks in the TBI models. It will be important to define what the impact of this therapy is on neuropathology. This is the first drug in OBTT targeting brain edema and it will be very enlightening to determine what the effect of reducing edema might be on behavioral and neuropathological outcomes.

Drug 7, Kollidan VA 64: Kollidon VA 64 is an agent categorized into the higher risk higher reward classification as outlined in our original OBTT grant application and publication.[90] It is also known by its

Chemical Structure of Kollidon VA 64.

chemical name vinylpyrrolidone-vinyl acetate copolymer and has the chemical structure shown. Kollidon VA 64 is used extensively as a vinylpyrrolidone excipient in the pharmaceutical industry. Although as a potential therapeutic agent in TBI, however, it is much more exploratory than the other agents tested thus far in OBTT. It is, nevertheless, potentially interesting in that it appears to have biological effects and in TBI results published to date suggest that it operates by a unique mechanism of action—via membrane resealing effects.[91] It is a large polymeric molecule with MWs being somewhat variable but ranging between 45,000 and 75,000. Thus, it is anticipated to only enter the injured brain in sites where there is substantial BBB permeability.

In the seminal pre-clinical study on this agent in TBI, IV administration of a single dose (500 microliters of a 1 mmol/L solution) at 1 h after CCI in mice significantly reduced acute cellular degeneration, BBB damage, brain edema, and motor deficits.[101] It also re-sealed injured cell membranes in brain tissue, but it did not appear that the ultimate benefit on secondary damage was a result of that mechanism—given that the cells exhibiting propidium iodide uptake ultimately went on to die whether or not they were in the treatment group. Kollidon VA 64 also attenuated caspase 3/7 activation. Of note, the effect of this agent of BBB permeability was remarkably large—almost completely ameliorating Evans Blue extravasation, and consistent with that finding, brain edema was reduced by >50% in treated vs. control groups. Thus other mechanisms conferring beneficial effects of Kollidon VA 64 appear to be operating. Finally, there is also some ongoing unpublished investigation on the mechanism of action of this agent and it may have effects as a Pannexin channel inhibitor—which may explain in part its "membrane resealing" effects—however that is still speculative and remains to be clarified.

In the published study, a lower dose of 250 µL of a 1 mmol/L solution was also shown to be effective (i.e., half of the aforementioned dose), and thus, it would seem that these two doses would be the most logical to pursue for this agent by the OBTT consortium. Regarding specifics of drug preparation, dosing and information beyond what is published, in personal discussion with the author of the seminal paper on this agent (Dr. Whalen, at Harvard Medical School), IP administration was not effective. In addition, the optimal way to prepare the agent is to dissolve 0.2 grams in 5 mL of sterile PBS and inject either 10 mL/kg or 20 mL/kg in the mouse ~3 or 6 mL, respectively, in a 300 gram rat. The concentration can also be doubled and to avoid administering 20 mL/kg of fluid which could alter our models, and it dissolves well in PBS, thus we will take that approach—since 6 mL is a fairly large volume. To ensure the capability of blinding treatment for this agent, we will prepare two different stock solutions of drug, 0.4 g in 5 mL of PBS or 0.2 g in 5 mL of PBS and always administer 10 mL/kg. Appropriately, the vehicle will be 10 mL/kg of sterile PBS. As in other studies in OBTT, the sham group will not receive treatment or vehicle.

Kollidon VA 64 was provided free of charge from BASF (Catalog # CAS-No 25086-89-9, Florham Park, NJ) as a powder to Dr. Kochanek who distributed it to the centers. Dr. Kochanek has communicated with technical support and the distribution teams at BASF and they are pleased to supply the agent to us. Based on the aforementioned publication and discussion with Dr. Whalen, it can be dissolved in sterile PBS (AMRESCO Biochemicals and Life Science Research Products, Catalog E504), which will also serve as the solution of the vehicle. The treatment groups included: 1) sham (surgery and catheters but no treatment), 2) vehicle (PBS) 10 mL/kg IV over 5 min, 3) low dose 10 mL/kg of a 0.2g/5 mL PBS solution IV over 5 min, and 4) high dose 10 mL/kg of a 0.4g/5mL PBS solution IV over 5 min. The therapeutic window for this agent is

suggested by the publication to be 1 h but to be consistent with our other acute therapies, and maximize its potential efficacy in a post treatment paradigm, we will once again give the treatment at 15 min after the insult. We also piloted administering the drug at the high dose to mice with arterial catheters in place to ensure that there was no adverse effect on blood pressure and it was well tolerated.   Please note that for these studies, as in our current OBTT paradigm, biomarker sampling is being carried out at 1 h, 4 h, 24 h, and a final time point identical to our most recent study.

All of the sites are working currently on this therapy, and almost all of the injuries and behavior have been completed for this agent—however, none of the codes have yet been broken, as per our protocol. Work on the neuropathology for Kollidon VA 64 is just commencing as it is being completed for Glibenclamide.

Drugs 8 and beyond: Our pharmacology team has developed an assay for minocycline (**Figure 16A-B**), to assess serum and brain levels and we are currently measuring levels in several dosing regiments. It is our next therapy to be tested and it will be administered by continuous IV infusion for 72 h in each animal. Our pharmacology team has been extremely helpful with regard to the study design and drug preparation and administration for each therapy tested, and includes Drs. Samuel Poloyac, Philip Empey, and Travis Jackson at the University of Pittsburgh. Subsequent to Minocycline we plan to test edaravone, amantadine, N-acetyl
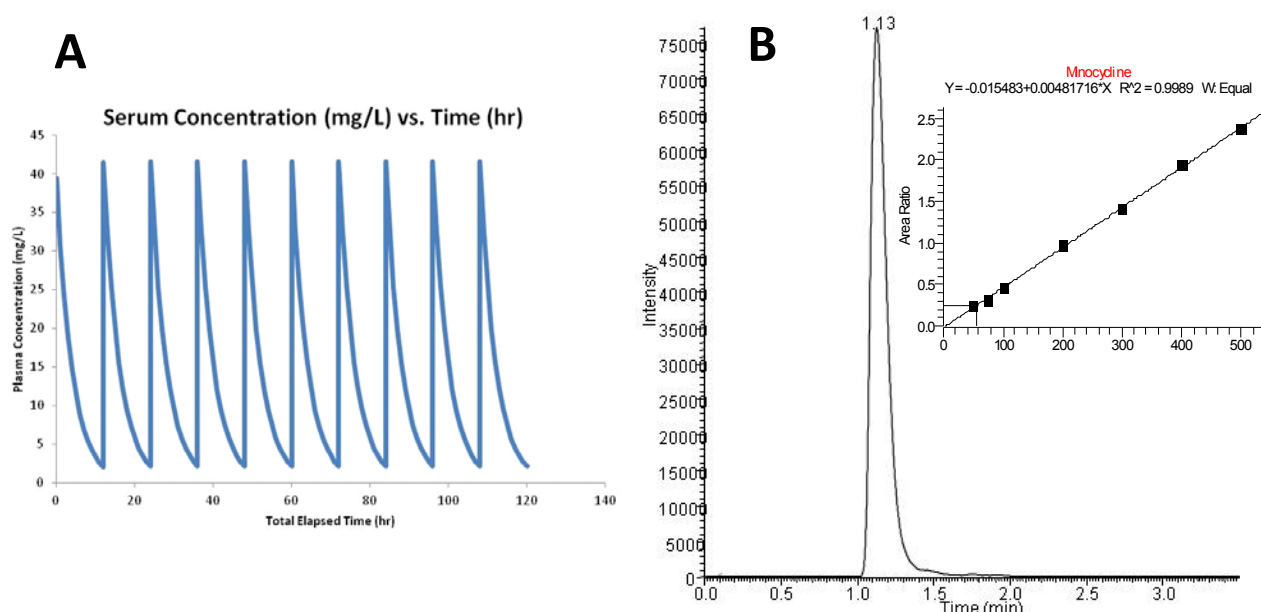

Figure 16A-B. Dose optimization tools for Minocycline which will be drug #7 in OBTT drug screening. Panel B shows the chromatographic assay to quantify Minocycline levels and its performance in the clinically relevant range of 50-500 ng/mL. Panel A shows the one-compartment predicted PK analysis for 30 mg/kg IV dosing in the rat. This approach could be extremely valuable to maximize therapeutic efficacy for some of the selected agents in OBTT Extended Studies.

cysteine amide (NACA), etanercept, and NIM 811 (if it is made available). Of note, we are –as indicated earlier in this proposal—also just launching OBTT-ES and the first more exploratory therapy will be AER-271 an aquaporin 4 receptor antagonist. That drug will actually precede minocycline. We have already put into place an MTA for the University of Pittsburgh to obtain and distribute this agent to Miami and WRAIR and just completed three pilots. More information on AER 271 will be available in the first report on OBTT-ES which represents a separate grant.

Serum Biomarker Development and Application to the primary screening studies: *Banyan Biomarkers (Ronald Hayes, PhD) and the University of Florida (Kevin Wang, PhD), Messina University (Stefania Mondello MD, PhD, MPH):* **This year, a total of 2412 sample analyses were run by Banyan** for GFAP and UCH-L1 for OBTT. This includes 1) all of the samples [N = 686 (1372 assays)] from the study of Simvastatin across the three rat models, 2) all of the samples [N = 484 (968 assays)] for the study of Levetiracetam across the three models, 3) assessment of an earlier time-point (1 h in addition to 4 h) across studies to determine if it improved performance of UCH-L1, and 4) a separate study [N = 18 serum and N = 18 plasma (72 assays)] comparing serum to plasma for GFAP and UCH-L1 that was carried out in the CCI model, given that in the CCI model, the

samples obtained and analyzed are from peripheral blood obtained through heparinized tubing rather than via a central catheter as obtained in the FPI and PBBI models. This does not appear to have had a major effect given the consistent findings that we have seen across the various studies within OBTT. Of note, to remedy this minor difference, Dr. C. Edward Dixon visited Dr. Tortella's laboratory and established an identical central venous catheter protocol for future studies in OBTT. That approach was taken in the VA-64 Kollidon studies that have been carried out in Pittsburgh. Finally, Dr. Mondello has been carrying out all of the statistical analyses and preparing all of the figures and tables for the biomarker data in the 5 treatment papers, and the cross model biomarker comparison manuscript. That represents a very major undertaking given all of the data in 6 manuscripts. Several examples of the cross model comparison findings are provided below.

Figure 17. Cross model comparison between blood levels of GFAP at 24h after either sham surgery or TBI in the 3 models in OBTT—assessed in rats in the vehicle groups from the first 3 treatment trials in OBTT. Significant increases vs respective sham were noted in each model. At 24h, the CCI model produced the greatest increase in GFAP (P<0.01 vs FPI and PBBI).

Figure 18. Cross model comparison of the relationship between blood GFAP levels and contusion volume in CCI (top), FPI (middle) and PBBI (bottom) assessed in rats from the first 3 drug trials in OBTT. An impressive and highly significant relationship was seen across models strongly suggesting excellent performance of GFAP in this regard.

Use of biomarkers of brain injury in rat blood (serum and/or plasma) to assess cross model comparisons, relationships of biomarkers to conventional behavioral and neuropathological outcomes in OBTT: The use blood (serum and/or plasma) biomarkers of brain injury in OBTT has yielded a number of outstanding findings. **Figures 17, 18, and 19** will be used as three representative examples, selected from a wealth of data that have been obtained on blood biomarkers. These examples typify the types of findings that OBTT is generating across these various areas of investigation using serum and/or plasma biomarkers. We have assessed both levels of GFAP and UCH-L1. Of note, GFAP has performed superbly in this regard and has shown excellent correlations across all 3 rat models with lesion volume, hemispheric or cortical tissue loss, MWM average latency, and probe trial performance. **Figure 17** shows relative comparison between models for 24 h GFAP levels, and **Figure 18** shows the correlations with lesion volume across models for GFAP. It is quite noteworthy that this performance is seen in **Figure 18** despite the fact that we –of course—did not vary the injury level within each of models. Rather blood levels of GFAP at 24 h were remarkably effective at detecting even the limited variance within a given model as it was used at an identical set of injury parameters in each rat. That finding is quite impressive and indicates that blood levels of GFAP are highly capable of predicting traditional behavioral and neuropathological outcomes and thus also have theragnostic potential in OBTT.
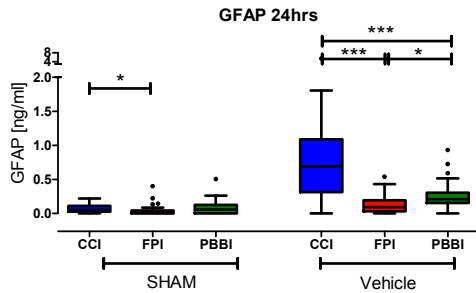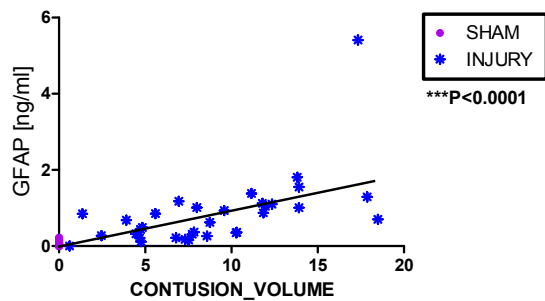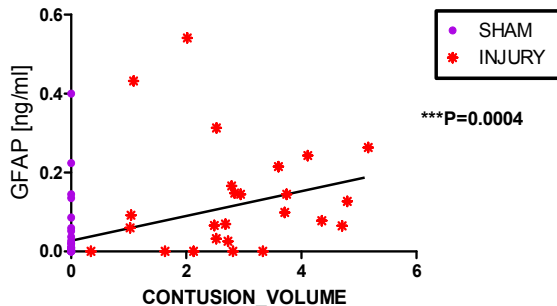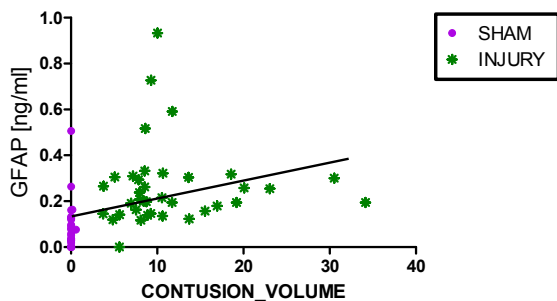
Figure 19 also serves as an example of how consistent the models are performing across the first 3 treatment trials in OBTT. In this example, we show sham and post TBI levels of GFAP at 4 h after either sham surgery or injury in the CCI, FPI and PBBI models (in rats treated with vehicle). As is evident, the model reproducibility as assessed by serum and/or plasma levels of GFAP at 24 h after injury was excellent across the 3 models used in drug screening in OBTT. GFAP levels are thus providing promising potential for theragnostic use of biomarkers across models and across drug testing in rat screening studies in OBTT. To further explore the theragnostic utility of blood biomarkers of brain injury in OBTT, we are, currently assessing how GFAP and UCH-L1 performed across models in each drug trial with specific attention to the Levetiracetam trial, which, as previously described, was the first therapy to show substantial benefit on cognitive outcome vs. vehicle in the FPI model, and an impressive reduction in hemispheric tissue loss in the CCI model. Those results should be available in the next report.

Work at Banyan is also ongoing with regard to assay development for GFAP and UCH-L1 in the micropig model and germane to this plan, samples have been sent to Banyan from Dr. Povlishock's laboratory at VCU including a time course study in his micropig injury model. **Finally, in response to the critique that we received last year, we have chosen to explore addition of a third biomarker to our studies, namely, the activated microglial marker iba-1. This work is being carried out at the University of Florida, under the direction of Dr. Kevin Wang, and is briefly outlined in the section below.**
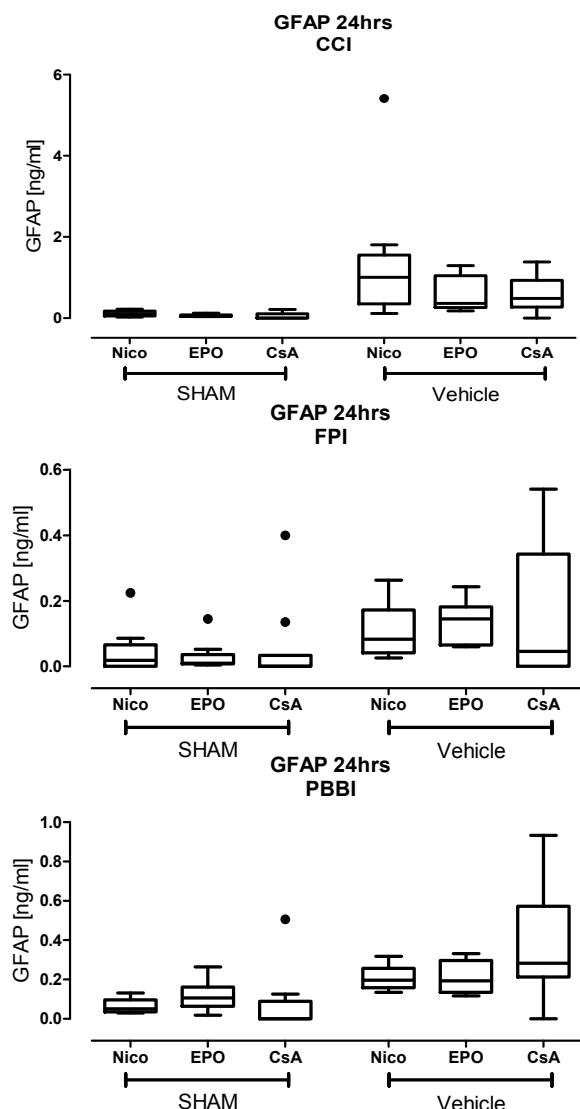
Microglial biomarker calcium-binding adapter molecule 1 (Iba-1). Iba-1 protein is encoded by the AIF1 gene in mammals. BA1 is a small protein (17,000 dalton). It belongs to the family of 4-EF-hand calcium-binding proteins. It is specifically expressed in microglia cells in the brain, although it is also present in macrophages in the peripheral compartments. Importantly Iba-1 is highly up-regulated during the activation of microglia cells upon brain injury or brain perturbation. Iba-1 is an actin-binding protein that is thought to play an important role in rearrangement of the plasma membrane actin-based cytoskeleton. Thus it has been suggested that up-regulation of Iba-1 protein during microglia activation plays a critical role in cytoskeleton dynamics, cell shape changes and cell migration.

Figure 19. Reproducibility of the 3 rat TBI models (CCI, FPI, and PBBI, in the top, middle, and bottom panels, respectively) in the first three drug studies in OBTT, namely nicotinamide (Nico), EPO, and CsA. Note that these data are from shams and rats treated with vehicle, so that the reproducibility of the models could be compared without confounders. The results strongly support the reproducibility of each of the models.

Microglial biomarker Iba-1 amplification-enhanced sandwich ELISA: A prototype Iba-1 assay is based on a proprietary amplification-enhanced ELISA method we developed in-house at Univ. Florida Center for Neuroproteomics & Biomarker Research (under Dr. K. Wang, **Figure 20,** below). Briefly, 96-well microtiter plates (Costar) were coated with capture MAb specific to Iba-1 (custom made). Following an overnight incubation at 40C, unoccupied binding sites were blocked for 1 h with casein. A 100 µl aliquot of diluted CSF or serum (dilution is used to avoid matrix effects) sample was added to the wells, incubated for 1 h and followed by the addition of a biotinylated-conjugated detection PAb specific to Iba-1 (custom-made) and further incubated for 2 h. After repeated washes with phosphate-buffered saline containing 0.2% Tween-20 (PBST), a proprietary set of streptavidin-coupled biochemical chain reaction reagents are added to achieve up to 1,000-fold amplification. Fluorescent, colorimetric or chemiluminescence signals are used as readout using a

microplate reader.   Iba-1 concentration in biosamples are calculated based on a standard curve using a range of Iba-1 antigen concentrations.

As a theranostic tool, Iba-1 may be responsive to therapies that suppress the neuroinflammatory process. We are excited and optimistic about that possibility. Towards this step, in the pig model of FPI, the VCU team led by Dr. John Povlishock has identified a robust Iba-1 induction in the corpus callosum and thalamus even at 6 h after injury (*please see elsewhere in this report*).

In the next funding year, we propose to first examine the compatibility of our above-stated Iba-1 ELISA system for pig Iba-1 detection; we will follow that by assaying Iba-1 in pig serum samples at various time points after injury (without drug intervention). There are available archived serum samples from ~ 26 pigs with pre-operation, and various acute post-injury time points (up to 6 h). The natural next step will be to examine if therapeutic intervention will affect the steady state or the kinetics of Iba-1 marker levels in blood in the pig TBI model. This assay may also have the potential of being crossed-used in the rat TBI models in the future which would add a third marker to the panel.
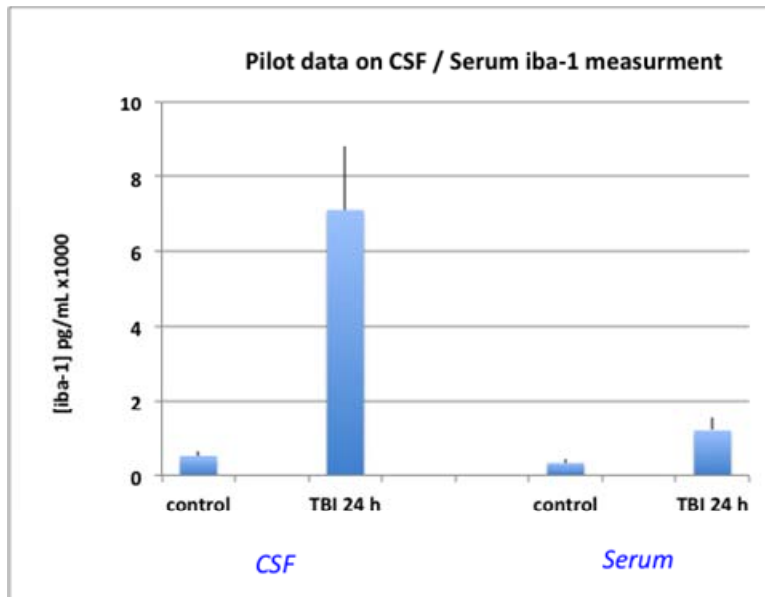

Figure 20. Examples of pilot results on assessment of iba-1 using human bio-samples: control and severe TBI CSF, and control and severe TBI serum (n=10 per group).

High visibility of Reportable Outcomes from the biomarker work in OBTT: The findings of cross model biomarker comparisons from OBTT have been selected for oral presentations thus far at the annual congress of the NNTS, the MHSRS, and the International Neurotrauma Society meeting. **In addition, the biomarker data will be one of the three featured panel presentations at the upcoming 2015 NNTS meeting. At these meetings, oral abstract selection is highly competitive. Thus, our biomarker findings and investigations are being viewed as highly important by the TBI community. We believe that they have substantial relevance to pre-clinical drug screening and also to better understand biomarkers in clinical use—since clinical TBI is often highly complex and heterogeneous.** This is an exciting development that has been apparent throughout the course of OBTT.

OBTT Studies in a large animal model of TBI: FPI in micropigs: *John Povlishock, PhD, Site PI, VCU:* Consistent with the expectations of this grant, 14 micropigs were critically evaluated from September 30, 2013 through September 29, 2014. All pigs were subjected to mild TBI involving central fluid percussion injury. A portion of this micropig population was equipped with cranial windows to assess vascular reactivity and all animals underwent detailed biomarker analysis, with serum samples harvested pre-craniotomy and post-craniotomy, as well as at 1min, 30 min, 1 h, 3h, and 6 h post-injury. All samples were prepared and processed consistent with the standards detailed by Banyan Biomarkers and the samples were forwarded to them for subsequent analyses. In those animals in which cranial windows were placed, multiple vascular assessments at the above-identified time points consistently demonstrated that the injury resulted in significantly diminished responsivity to known vasodilators such as acetylcholine. In those animals assessed, this vascular impairment persisted over the 6 h window of evaluation. Following the completion of these vascular studies at 6 h postinjury, the animals were perfused and their brains prepared for detailed immunocytochemical analysis. APP immunocytochemistry was used to quantitatively assess the burden of any associated axonal damage found within the corpus callosum and thalamus. All animals analyzed to date revealed a consistent, high yield of damaged axons per unit area within the same loci in the corpus callosum and thalamus, thereby allowing for rigorous quantitative assessment of any targeted therapy identified by the OBTT study group (**Figure 21**).
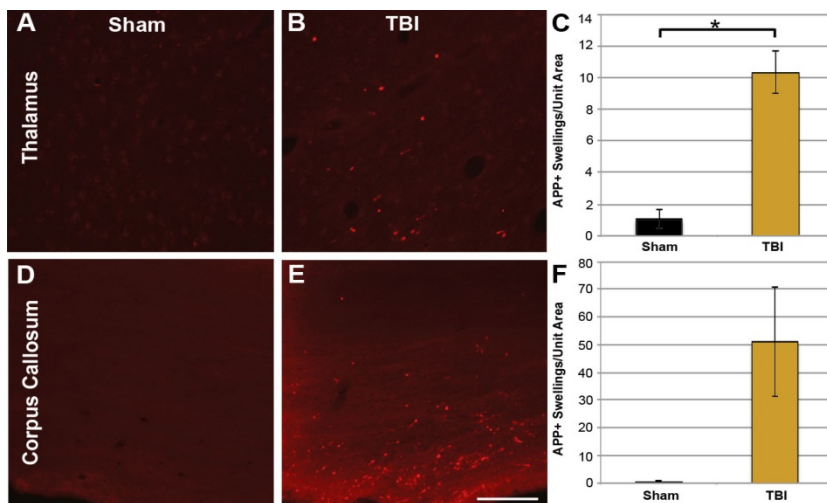
Figure 21. Representative photomicrographs of amyloid precursor protein (APP) immunofluorescence from animals sustaining **A&D** sham (n=3) or **B&E** CFPI (n=18) in the **A&B** thalamus and **D&E** corpus callosum (CC). Bar graphs depicting the average number of APP labeled axonal swellings in the **C** thalamus and **F** CC in a 0.72mm² area. DAI was quantified using systematic random sampling in conjunction with the particle analysis tool for ImageJ (thalamus:10images/section, 6sections/pig; CC:2images/section, 12sections/pig). Mean±SEM. * p<0.05. Scale bar:200µm.

In concert with these quantitative analyses, detailed immunocytochemical analyses were also conducted to evaluate UCH-L1, GFAP, and Iba-1 immunoreactivity, with some samples double labeled for 3D reconstruction of the obtained confocal images. Although these studies are not fully complete, a detailed picture is emerging which shows that the sites of APP+ axonal damage correlate with a dramatic upregulation in Iba-1 microglial immunoreactivity that maps directly to these loci . In contrast, the non-axotomized brain regions show no evidence of Iba-1 upregulation. (**Figure 22**). In addition to these findings, it is also of note that in the thalamus, the upregulation of Iba-1, together with a finding of APP+ damage, also correlates with a dramatic upregulation of neuronal UCH-L1 immunoreactivity. Parallel analysis of GFAP awaits further quantitative assessment. Lastly, to confirm the localization of these immunocytochemical reaction products and their overall relation to axotomy and/or neuronal death or perturbation, parallel ultrastructural analyses were performed. These studies confirmed the diffuse nature of the axonal injury while also confirming that the brain regions assessed contained no evidence of direct neuronal or glial cell death, despite upregulation of the factors identified above.
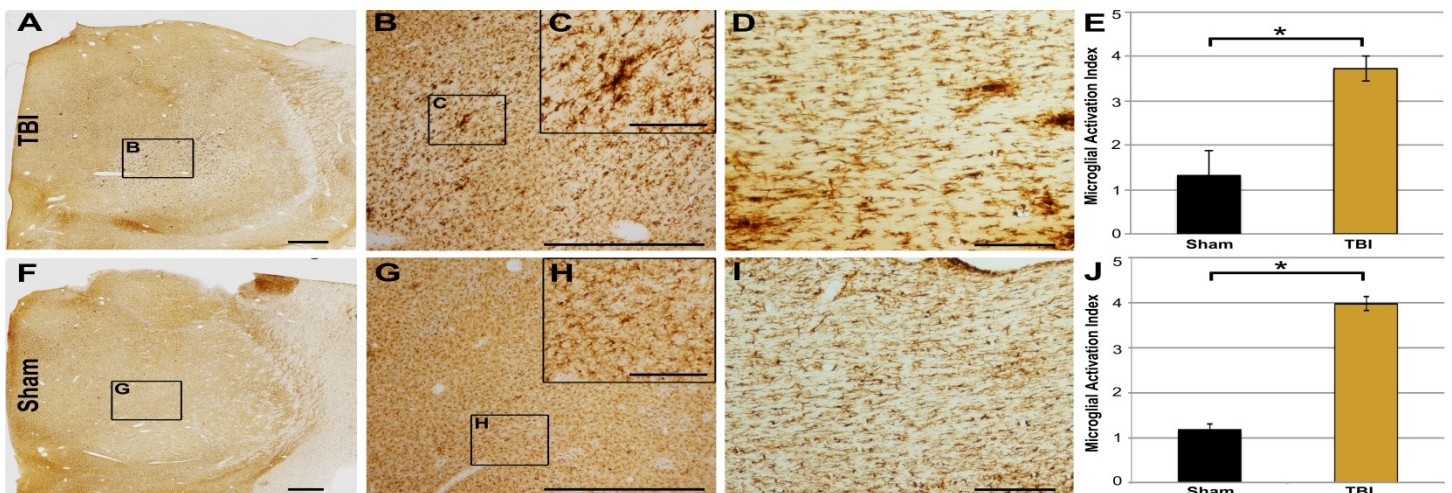

Figure 22 Representative photomicrographs of the microglial marker Iba-1 in the **A-C & F-H** thalamus or **D & I** corpus callosum (CC) of **A-D** CFPI (n=18) and **F-I** sham (n=1) pigs. **B&C** are magnified regions indicated in **A&B** and **G&H** are magnified regions indicated in **F&G** respectively. Note that the microglia appear ramified, indicating a quiescent state in sham-injured animals. While ramified microglia are present in brain injured pigs, a large proportion of microglia have retracted, amoeboid or stellate morphologies in the thalamus, and retracted and/or bushy morphologies in the CC, indicating activation. **E&J** Bar graphs illustrating the degree of microglial activation in the **E** thalamus and **J** CC as quantified by two blinded investigators using a scale from 0-5 (0=no activated microglia, 5=extreme microglial activation). Scale bar **A&F**=1mm, **B-D & G-I**=200µm. Mean ±SEM. * p<0.05.

Other accomplishments by the OBTT consortium in year 4:

1. The OBTT consortium investigators are working on 8 manuscripts that will comprise a special issue of the *Journal of Neurotrauma* reporting the results of the first 5 therapies, the biomarker work thus far in OBTT, and both introductory and concluding manuscripts.

2. Dr. Kochanek represented OBTT at the recent US Army Neurotrauma Pharmacology Workshop, which generated a comprehensive document for the Army on TBI pharmacology that was just published in the *Journal of Neurotrauma*.
3. Dr. Kochanek submitted a comprehensive review manuscript on Emerging Therapies in TBI for a special issue of Seminars in Neurology that is being edited by COL Geoff Ling.
4. At the Safar Center site a number of pilot and preliminary studies were carried out in mice and rats to explore potential new therapies (such as therapies to target edema via blocking HMGB1, among others), define specifics related to dosing and drug levels for the various therapies, and also to begin to test Levetiracetam and Glyburide among other promising therapies in our murine model of combined TBI plus hemorrhage – as specifically outlined in the grant proposal. Of note, the Safar Center site serves as a special resource for OBTT to probe and/or optimize therapies prior to launching them across the entire consortium – which represents a huge and expensive undertaking that must be optimally orchestrated. Given the size of this document the details of these pilot and preliminary studies are not provided. Nevertheless, they are important to the success of OBTT.

KEY RESEARCH ACCOMPLISHMENTS Since THE INCEPTION OF OBTT—Accomplishments for this funding year (ongoing or new) are bolded for convenience of the reviewer.

1. **IACUC and ACURO Approval at all sites along with necessary updates**
2. **Creation and continual updating of an Operations Manual for the OBTT consortium by Dr. Kochanek**
3. **Monthly consortium investigator conference calls**
4. **TBI drug therapy literature review, investigators survey, and selection of therapies to be evaluated by the OBTT consortium (ongoing)**
5. **Comprehensive review of the TBI literature for the first nine drugs, nicotinamide, EPO, CsA, Simvastatin, Levetiracetam, Glibenclamide, Minocycline, NIM-811, Edaravone, Kollidon VA 64, Etanercept, Amantadine, and NACA by Dr. Kochanek, with updating of the manual through the most current agent (IACUC and ACUROs either submitted or approved at all sites).**
6. **Publication of 3 manuscripts on 1) the OBTT concept in the *Journal of Trauma,* and on therapy reviews germane to the US Army (see Reportable Outcomes 1, 26, 27)**
7. **Biomarker assessments of >1000 rat samples.**
8. **Biomarker assay development for micropig assays of GFAP and UCH-L1**
9. **Presentation of 38 abstracts and/or National or International presentations since the inception of OBTT including 14 in year 4**
10. Report sent by Dr. Kochanek on the launching of OBTT to the Therapy and Oversight Committee and Consultants
11. **Completion of all experiments for drugs #1 (nicotinamide), #2(EPO), #3 (CsA), #4 (Simvastatin), #5 (Levetiracetam), #6 (Glibenclamide), and ongoing studies with #7 (Kollidon VA 64) and just initiating #8 Minocycline—in primary screening across three rodent models with ~1000 rats studied.**
12. **Investigators meeting held on at the 2011-14 National Neurotrauma Society Meeting**
13. **Presentation of symposia on OBTT by the PI and site PIs at the 2011-14 ATACCC/MHSRS conferences, including plenary lectures on OBTT by the PI at the 2012 ATACCC and 2014 MHSRS conferences.**
14. Presentation by the PI of a plenary lecture on OBTT at the 2012 annual meeting of National Neurotrauma Society. **A Panel session on OBTT was accepted for presentation at the 2015 NNT Congress.**
15. **Re-establishment and continued refinement of the large animal micropig model of FPI TBI at Virginia Commonwealth University with biomarker studies completed, and studies with Levetiracetam therapy planned for 2015.**
16. **Dr. Kochanek represented OBTT at the US Army Neurotrauma, Pharmacology Work Group. He was the second author of the comprehensive document generated by that group and recently published in the *Journal of Neurotrauma*.**

17. Preparation of a full grant application titled Operation Brain Trauma Therapy-Extended Studies requested by CCCRP. Dr. Kochanek prepared the application. **The application was funded.**
18. **Ongoing preparation of 8 manuscripts by the OBTT investigators for invited submission as a special issue of the *Journal of Neurotrauma* devoted to OBTT. 4 have been completed.**

REPORTABLE OUTCOMES (All reportable outcomes since project inception are shown, those from the 2013-2014 funding year are shown in bold font)

1. Kochanek PM, Bramlett H, Dietrich WD, Dixon CE, Hayes R, Povlishock J, Tortella F, Wang K: A novel multi-center pre-clinical drug screening and biomarker consortium for experimental traumatic brain injury: Operation Brain Trauma Therapy. J Trauma 71(1 Suppl):S15-24, 2011.

2. Kochanek PM, Dixon CE: Operation Brain Trauma Therapy (OBTT) Consortium: Program Overview/University of Pittsburgh Program. Presented at the ATACCC Meeting, Ft. Lauderdale, Florida, August 15-19, 2011.

3. Bramlett HM, Dietrich WD. Operation Brain Trauma Therapy (OBTT) Consortium: University of Miami Miller School of Medicine Program. Presented at the ATACCC Meeting, Ft. Lauderdale, Florida, August 15-19, 2011.

4. Shear DA, Schmid KE and Tortella FC. Operation Brain Trauma Therapy (OBTT) Consortium: The WRAIR Program (Penetrating Ballistic-Like Brain Injury). Presented at the Advanced Technology Applications to Combat Casualty Care (ATACCC) Conference in Fort Lauderdale, FL, 2011.

5. Povlishock, JT. Operation Brain Trauma Therapy: The Virginia Commonwealth University Program. Presented at the Advanced Technology Applications to Combat Casualty Care (ATACCC) Conference in Fort Lauderdale, FL, 2011.

6. Kevin K.W. Wang, Ronald L. Hayes Operation Brain Trauma Therapy (OBTT) Consortium: Banyan Biomarkers Core. Presented at the Advanced Technology Applications to Combat Casualty Care (ATACCC) Conference in Fort Lauderdale, FL, 2011.

7. Kochanek PM: Operation Brain Trauma Therapy (OBTT). Oral plenary presentation, 2012 Congress of the National Neurotrauma Society, Phoenix, AZ, July, 2012.

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CONCLUSION

The unique multicenter pre-clinical drug screening consortium OBTT continues to be highly productive, has nearly completed all studies on 7 therapies and has identified a therapy that has shown benefit in 2 models, namely Levetiracetam. just In addition, the serum biomarker GFAP has also shown considerable promise as a diagnostic and theragnostic for pre-clinical work. We have a begun beginning to investigate our 8th therapy. Finally, OBTT is garnering national and international recognition at multiple conferences and a special issue of issue of the Journal of Neurotrauma on the findings of OBTT is in preparation.

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